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THE EFFECTS OF GROWTH, ANAEROBIC (SPRINT RUNNING), AND AEROBIC (CONTINUOUS RUNNING) TRAINING ON PROTEIN, NUCLEIC ACIDS AND CONNECTIVE TISSUE IN THE LEFT VENTRICLE OF MALE RATS

by

NORMAND GIONET

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSICAL EDUCATION

EDMONTON, ALBERTA

FALL, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled: "The Effects of Growth, Anaerobic (Sprint Running), and Aerobic (Continuous Running) training on Protein, Nucleic acids and Connective tissue in the left ventricle of male rats", submitted by Normand J. Gionet in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physical Education.

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supervisor

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Denis Massicotte

External Examiner

Date October 14, 1977

DEDICATION

A mon épouse Shaaron, son amour,

sa confiance, son dévouement, sa compréhension

ABSTRACT

The present study was designed to investigate the effects of growth, anaerobic (high intensity sprint running) and aerobic (moderate intensity continuous running) training on protein, nucleic acids and connective tissue in the left ventricle of male rats. Ten 5-week-old male rats were used as basic or initial control group and ninety (90) male rats of which 3 groups of thirty (30) animals; each group consisted of: 1- age-matched control groups (C14, C25 and C33); 2- the anaerobic trained groups (AN14, AN25 and AN33); 3- the aerobic trained groups (A14, A25 and A33). The attrition rate of the present study was 19% of which 4% occurred in the control groups, 7% and 8% of the anaerobic and aerobic trained groups respectively. The so-called anaerobic training program consisted of intermittently sprinting on a treadmill set at 30% grade, at high intensity for 15 seconds interspersed by 20 seconds rest repeated ten times twice a day, 4 days a week. The animals ran at initial speed of 40m/min. to final speeds of 80, 85 and 90m/min. after 9, 20 and 28 weeks of training respectively. The so-called aerobic training program consisted of continuously running on a treadmill set at 8% grade, at moderate intensity for 30 minutes twice a day, 4 days a week. They ran at an initial speed of 15m/min. to final speeds of 30m/min. after 9 weeks of training and 35m/min. after 20 and 28 weeks of training. Significant growth alterations were found for each variable measured except for the RNA to DNA ratio and RNA

concentration, in animals 5 to 14 weeks of age. The magnitude of growth change was more pronounced in pre-pubertal animals (5-14 weeks of age) as compared to mature animals (25-33 weeks of age). Body weight and hydroxyproline content were decreased significantly following 9 weeks of aerobic training while the adjusted heart weight, DNA concentration, weight per nucleus and also body weight were altered significantly following 20 weeks of aerobic training. All the variables measured in the anaerobically trained groups were not affected following 9, 20 and 28 weeks of training. The significant increase in the adjusted heart weight in addition to significant increase and decrease in the DNA concentration and weight per nucleus respectively in the left ventricle, would seem to imply that cardiac hypertrophy, if not left ventricular hypertrophy were induced after 20 weeks of aerobic training. Although the adjusted left ventricular weight of the 20-week aerobic trained group was not significantly different from the age-matched control, the tendency towards a larger left ventricle was nevertheless apparent. The observed results seemed to indicate a consistent trend towards a greater improvement in some of the cardiac parameters investigated following 20 weeks of aerobic training. Further aerobic training does not seem to overload sufficiently the cardiac muscle to adapt above the already attained functional level.

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INTRODUCTION

The manner in which the intact organism responds to exercise has intrigued cardiovascular physiologists for the past century.

The cardiac adjustments, necessary to meet the demands of the working muscle remain areas of intense investigation and speculation.

However, physical training is thought to have a beneficial effect on the overall functioning of the cardiovascular system. This is based on both epidemiological (Fox and Skinner, 1964) and on experimental (Saltin et al., 1968) studies in humans. Even though most studies on the cardiac adaptation to exercise in small animals are only in their infancy, numerous investigations during the past decade have been published (see for reviews: Barnard, J., 1973; Vatner and Pagani, 1976).

Several investigators have reported that severe, prolonged exercise induced cardiac enlargement in small animals (Crews and Aldinger, 1967; Oscai et al., 1971 a,b; Penpargkul and Scheuer, 1970; Bloor and Leon, 1970; Jaweed et al., 1974; Steil et al., 1975). This is reflected by an increase in the myocardial wall thickness which permits the left ventricle to meet the increased wall tension that has resulted from the increase radius of curvature (Ford, 1976). This increase in mass is considered an important adaptive mechanism which enables the heart to adjust itself to chronic

work and energy requirement (Young, 1970; Rabinowitz, 1973; Ford, 1976). This enlargement in physiologic hypertrophy is due to an increase in myocardial cell size without alteration in myocardial cell numbers (Linzbach, 1960; Crews and Aldinger, 1967; Ford, 1976).

This structural alteration, induced by exercise increases the cardiovascular reserve of exercised animals (Crews and Aldinger, 1967; Penpargkul and Scheuer, 1970; Rushmer, 1976).

This adjustment of cardiac mass and of cardiac muscle cell size to chronic volume overload of exercise would imply that a net synthesis of proteins and nucleic acids have taken place. However, following chronic exercise, the myocardial protein, DNA and RNA contents have been shown not to increase above normal levels (Bell et al., 1975; Dowell et al., 1976 a,b; Sordahl et al., 1977).

During exercise the untrained and trained animals can increase the cardiac output above resting levels to meet the varying metabolic requirement of the whole body. In the untrained animals, this is provided by increasing the heart rate (tachycardia) with small changes observed in the force of contraction (stroke volume). On the other hand, the trained animals due to the increase in ventricular mass resulting from chronic volume overload during exercise increase their cardiac output by increasing the inotropic state of the heart, i.e. the force of contraction (Ford, 1976). This adaptation is reflected in a lower resting heart rate (bradycardia)

commonly observed in trained animals (Barnard et al., 1976). The increased force of contraction of the heart has also been observed in isolated perfused heart from trained animals with cardiac hypertrophy (Crews and Aldinger, 1967; Penpargkul and Scheuer, 1970).

The biological significance of cardiac hypertrophy that develop during strenuous and prolonged muscular activity seems to be to provide a greater maximum cardiac output (greater reserve of heart rate and stroke volume) and contribute, among other adaptative changes, to the superior performance of the active skeletal muscle of the trained animals. Therefore, the heart adapts to the circulatory load by increasing its mass to meet the varying demands of the body during exercise, especially the exercising skeletal muscle.

The myocardial fibers are supported and are firmly attached to collagen forming the "skeleton" of the heart (Rushmer, 1976). The collagen is believed to be exclusively synthesized by the connective tissue (Skosey et al., 1972) and is known to be distributed primarily between muscle fibers and along blood vessels (Spann et al., 1971).

During experimentally induced cardiac hypertrophy, the connective tissue is increased considerably (Morkin and Ashford, 1968; Grove et al., 1969 b; Meeson, 1971) and the bulk of the DNA synthesis seems to be associated with mitotic activity mainly in the connective tissue cells (Nair et al., 1971).

In physiological induced cardiac hypertrophy, the

content of collagen was also increased in young rats after a severe and prolonged endurance exercise (Bartosova et al., 1969; Chvapil et al., 1973). Since the collagen in distributed between blood vessels, the observed increase could reflect the normal distribution of the coronary vasculature which can also be increased by chronic exercise (Tepperman and Pearlman, 1961; Stevenson et al., 1964), and could tend to give greater support to the myocardial fibers during contraction.

There seems to be a general recognition among investigators that biochemical and structural adaptations of the myocardium are observed following physical exercise and that such changes are beneficial to an enhancement of the capacity of the myocardium to do work.

Most of the reported literature studying the effects of physical training on the cardiac muscle have commonly used an endurance kind of exercise, i.e. at moderate intensity, running continuously for a limited period of time.

Baldwin et al., (1977) have studied for the first time of differential effects of two types of training program on the cardiac muscle of female rats. The training programs consisted of a high intensity interval running and a moderate intensity continuous running extended over 10 weeks. The high intensity interval training program increased significantly the ventricular weight and myofibrillar ATPase whereas no change in these variables was observed after the continuous training program. These findings suggested that a higher

degree of stress applied to the cardiac muscle of the female rats by the high intensity interval running was responsible for bringing about these adaptations. Such changes could provide the heart with a greater potential for increasing stroke volume via changes in its contractile potential.

In the investigation of Hickson et al. (1976), they have used very high intensity (sprint) running for their training program and have looked at the heart muscle. They have trained their male rats to run on a controlled-running wheel at 99m/min., 5 days per week for 8 weeks. The intermittent running program consisted, after the 37th day of training, of completing 8 bouts spaced by 2.5 minutes of inactivity between them. Each bout consisted of 6 repetitions of running for 10 seconds followed by a rest of 40 seconds. This very high intensity of running produced a significant decrease in the body weight of the exercised rats. While the absolute heart weight of the exercised rats was not found to differ from the control, the relative heart weight was greater in the exercised group when compare to the control group due to the reduction in body weight. These findings are similar to those reported after endurance training of moderate intensity (Oscai et al., 1971 a; Jaweed et al., 1974; Houston and Green, 1975; Dowell et al., 1976 a).

PURPOSE OF THE STUDY

No study at the present time has been conducted to attempt to differentiate the effects of an anaerobic and an aerobic training programs extended over several months, on

selected cardiac parameters in the left ventricle of male rats.

With these observations in mind, the aims of the present study are twofold:

1- to determine the differential effects of a high intensity (anaerobic) running and of a moderate intensity (aerobic) running on selected cardiac parameters in male rats;

2- to determine the effects of such training programs on selected cardiac parameters at different stages of growth and development of male rats.

DEFINITION OF TERMS:

1- Adaptive hypertrophy: increase in size of an organ in response to changed conditions, eg. increase in the wall thickness of the ventricles due to training;

2- Aerobic training: exercise stressing the energy-yielding system of aerobic metabolism of the animals, also known as endurance training;

3- Anaerobic training: exercise stressing the energy-yielding system of anaerobic metabolism of the animals, also known as high intensity (sprint) training;

4- Cardiomegaly: denotes an increase in the mass of cardiac tissue in pathological (Badeer, 1972) as well as in physiological (Crews and Aldinger, 1967) hypertrophy;

5- Concentric hypertrophy: increased thickness of the walls of an organ characteristic of all physiological

enlargement;

6- Eccentric hypertrophy: hypertrophy of a hollow organ with dilatation of its cavity and wall thickness: characteristic of pathological hypertrophy;

7- Heart ratio: the heart weight divided by the body weight;

8- Hydroxyproline: contains the amino acid, proline which is usually found in collagen. The measurement of hydroxyproline makes it possible to determine the collagen and thus the connective tissue of the particular organ;

9- Hyperplasia: an alternative mode of growth consisting of the multiplication or increase in the number of normal cells or fibers of an organ;

10- Hypertrophy: another alternative mode of growth consisting of the enlargement or overgrowth of an organ or of an increase in size of the cell constituents;

11- Ontogeny: the development of the individual organism;

12- Pathological hypertrophy: increase in the mass of an organ due to an increase in cell number as well as an increase in cell size induced by hypertension and/or valvular disease (Crews and Aldinger, 1967);

13- Physiologic hypertrophy: increase in the mass of an organ due to an increase in cell size rather than an increase in the cell number produced by physiologic activity, eg. exercise (Crews and Aldinger, 1967);

14- Protein to DNA ratio or protein per nucleus:

represents the relative content of protein per cell and gives some indication of cell size (Winick and Noble, 1965);

15- RNA to DNA ratio or RNA per nucleus: indicates the content of RNA per cell of an organ;

16- Total number of nuclei: according to Enesco and Leblond (1962), it represent cell number assuming a constant DNA content in a single diploid nucleus;

17- Weight per nucleus: a relatively good indication of the cell size of an organ according to Enesco and Leblond (1962);

ABBREVIATIONS

DNA: deoxyribonucleic acid;

RNA: ribonucleic acid.

THE EXPERIMENTAL MODEL: THE RAT

In the past decade or so, the field of Physical Education has opened up to many new area which consisted of studying man in motion from the point of view of science. Such field of investigation comprises the area of the physiology and/or the biochemistry of exercise.

Their main purposes is to conduct experiment on animals and to look more closely at the effects of exercise at the cellular level. Such objectives can be realized with the use of animals.

However, the ultimate objective of animal research in the field of exercise physiology and biochemistry for the researcher, would be an attempt to interpret the significance

of the result to humans. An objective that is often forgotten by many researcher.

The advantages and/or disadvantages of animal research in the field of Physical Education are listed below.

THE ADVANTAGES

1- One of the main feature of conducting animal research is the accessibility and the ease of handling and controlling of such experimental subjects. In other words, they are there when you want them.

2- The response of the animals to different treatments can easily be controlled because of the similarity of their genetic make-up. Coming from the same strain, the animals used, are usually the result of 20 generations.

3- The use of invasive technique makes it easier to thoroughly investigate different parts of the animal and therefore opens up different channels of study. This will permit the research with a much more understanding of the response of different physiological and biochemical parameters under the stress of exercise.

4- Animals are good experimental models to investigate the effects of unknown phenomena or treatments (such as drugs) before it is even tried on humans.

THE DISADVANTAGES

1- Experimental data resulting from animal studies make it difficult to generalize to the human population. But in fact, it can give some perspective of how it would

affect man.

2- The effects of different treatments given to animals might not have the same effects on man. This obviously depends of the kind of treatment employed. But in reality, it is something that researcher have to speculate on.

3- Keeping animals in a restricted area, (such as in small cages) might not be comparable to a normal environment for such animals. In other words, especially in exercise studies, the control animals might, in fact, be the experimental animals.

4- If researchers are interested in the study of man in motion, and if they are interested to promote knowledge and facts about the effects of exercise on man, they should be conducting their research on humans with the use of proper technique (eg. muscle biopsies), and not with animals.

The whole field of animal research in the field of Physical Education lies in the philosophy of the researcher. In other words, he has to ask himself the question: What benefit it would have on the advancement of the study of man in motion, if he was to conduct experiments using animals?

METHODOLOGY

I- ANIMAL CARE AND SELECTION

A total of one-hundred male rats* ((WOF (WI), specific pathogen free) weighing approximately ninety to one-hundred grams were obtained through the Director of the Health Animals Science Center at the University of Alberta. Upon arrival, the four week-old rats were housed in individual cages. The air-conditioned room was maintained at a temperature of 22°C and lighted from 1600 to 0600 hours. All animals were fed a normal protein diet (23% crude protein) of Purina rat chow (Appendix A-I) and watered ad labitum throughout the entire study.

Previously marked cages served the purpose of randomly selecting the animals for each particular group. Forty (40) rats consisted of the control groups and sixty (60) rats the training groups, i.e. thirty (30) rats in the interval (sprint) running group (anaerobic) (Table 1). These two latter groups were age-matched with the control animals and trained for 9, 20 and 28 weeks.

The two training groups were designated as primarily aerobic or primarily anaerobic, and these terms were used to differentiate between the two groups constantly throughout the study.

*The attrition rate of the present study was 19% from which 4%, 7% and 8% consisted of the control, anaerobic and aerobic groups respectively. Therefore, the data were obtained from the 81 remaining rats.

TABLE 1 - Control and Trained Animals, Code, Number of Animals, Training Times and Age at Time of Sacrifice.

Animals Groups	Code	Number of Animals (N)	Training Times (weeks)	Age at Time of Sacrifice (weeks)
BASIC CONTROL GROUP (no training)	BC5	10	-	5
CONTROL GROUP (age-matched with AN14 and A14)	C14	9	-	14
ANAEROBIC GROUP (sprint training)	AN14	8	9	14
AEROBIC GROUP (endurance training)	A14	9	9	14
CONTROL GROUP (age-matched with AN25 and A25)	C25	10	-	25
ANAEROBIC GROUP (sprint training)	AN25	9	20	25
AEROBIC GROUP (endurance training)	A25	7	20	25
CONTROL GROUP (age-matched with AN33 and A33)	C33	7	-	33
ANAEROBIC GROUP (Sprint training)	AN33	6	28	33
AEROBIC GROUP (endurance training)	A33	6	28	33

Before the commencement of the training program, all the animals were given a one week cage confinement so as to provide a suitable adjustment to the new environment. They were handled during that week and this practise continued throughout the experiment. Daily rotation of the cages allowed the animals to change position such that no animals

remained in the same place on consecutive day. Cages were washed and sterilized once a week while the trays beneath them were cleaned and swabbed once a day. Food was replenished when needed and fresh water changed every second day.

II- ORIENTATION ON THE TREADMILL

At the beginning of the second week following their arrival, the experimental groups (anaerobic and aerobic groups) were oriented on a motor driven treadmill (Quinton rodent treadmill) so as to become accustomed to it.

The treadmill consisted of a wide endless belt on rollers divided into ten compartments (48 x 9.5cm) suspended over the belt. This provided sufficient space for each animal to run. Motivation was reinforced by an electric shock (50 Volts) which was fitted on a grid at the rear of each compartment. The animals learned to keep pace with the belt movement by the electrical stimulation (Holloszy, 1967).

III- TRAINING PROGRAM

Two types of training programs were used in this study, i.e. an endurance (aerobic) program and a high sprint running (anaerobic) program. One group of animals was trained for a period of 9 weeks while two other groups trained for a period of 20 and 28 weeks respectively.

The soecalled aerobic program consisted of continuously running at moderate intensity for thirty (30) minutes at 30-35 meters per minute on the treadmill set at

an 8% grade. This program was performed in the morning and in the afternoon four times a week (i.e. Monday, Tuesday, Thursday and Friday) (Appendix A-II).

The so-called anaerobic program consisted of intermittently sprinting for fifteen (15) seconds interspersed by twenty (20) seconds rest repeated ten (10) times at an average speed of 75 meters per minute (ranged between 40 meters/min. to 90 meters/min. throughout the study) on the treadmill set at a 30% grade. This program was also performed in the morning and in the afternoon, four times a week (i.e. Monday, Tuesday, Thursday and Friday) (Appendix A-III).

The animals in both training groups were trained by strictly adhering to the running programs outlined in Appendices A-II and A-III. The total distance (in meters) that was covered during the training programs is outlined in Appendix A-IV.

The selection of the two types of training program was an attempt to stress the cardiac muscle at different work loads, i.e. specific training programs preferentially stressing either the aerobic or the anaerobic energy-yielding systems of the skeletal muscle of the animals.

IV- EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A 3 x 3 factorial design (fixed model) was used in the present study (Keppel, 1973). The three levels of factor A (treatments) consisted of the control groups (C14, C25 and C33), the anaerobic groups (AN14, AN25 and AN33), and aerobic groups (A14, A25 and A33). The three

levels of factor B were the extent of the running program in weeks and consisted of the 14-week-group (or 9 weeks of training), the 25-week-group (or 20 weeks of training), and the 33-week-group (or 29 weeks of training).

A two-way analysis of variance was used to determine which, if any, main effects were significant between treatments (factor A), between training time and/or growth (factor B) and also if any interaction (AB) effects were significant as outlined in Table 2.

TABLE 2- A Two Way Analysis of Variance Table for Comparison of Group Means.

		FACTOR B		
		9 WEEKS OF TRAINING	20 WEEKS OF TRAINING	28 WEEKS OF TRAINING
FACTOR A	CONTROL	C14 (9)[a]	C25 (10)	C33 (7)
	ANAEROBIC	AN14 (8)	AN25 (9)	AN33 (6)
	AEROBIC	A14 (9)	A25 (7)	A33 (6)

a- Number of Observations in each cell.

If significant F-ratios were obtained, a one-way analysis of variance between groups and time of training was executed to test for simple main effects. If the one-way analysis of variance was significant, a Student Newman-Keuls (SNK) test was used as comparison between individual means.

A one-way analysis of variance was also employed to see whether the various changes in the cardiac parameters as a result of growth (between 5-week-old animals (BC) to 33-week-old animals (C33) was significant. In addition, a similar analysis was conducted between the basic control and the experimental groups so as to investigate the training effects on the developmental patterns of the parameters under study.

The dependent variables investigated in this study are listed below:

1- BODY WEIGHT in grams (gm);

2- HEART WEIGHT in milligrams (mg);

3- ADJUSTED HEART WEIGHT in mg;

4- LEFT VENTRICULAR WEIGHT in mg;

5- ADJUSTED LEFT VENTRICULAR WEIGHT in mg;

6- TOTAL Left Ventricular PROTEIN in mg;

7- TOTAL Left Ventricular DNA in micrograms (ugm);

8- TOTAL Left Ventricular RNA in ugm;

9- TOTAL Left Ventricular HYDROXYPROLINE in ugm;

10- LEFT Ventricular PROTEIN CONC. in mg/gm of wet weight;

11- LEFT Ventricular DNA CONC. in mg/gm of wet weight;

12- LEFT Ventricular RNA CONC. in mg/gm of wet weight;

13- LEFT Ventricular HYDROXYPROLINE CONC. in mg/gm of wet weight;

14- LEFT Ventricular RNA to DNA RATIO;

15- LEFT Ventricular PROTEIN to DNA RATIO;

16- Total NUMBER OF NUCLEI in the Left Ventricle (in millions);

17- WEIGHT PER NUCLEUS of the Left Ventricle in nanogram (ngm).

The dependent variables as listed, were statistically analysed from the Statistic Package for the Social Sciences (S.P.S.S. ANOVA) programs according to Nie et al., 1975. Significant differences were accepted at the alpha (P) 0.05 where P was the probability that no differences existed between means. Thus in the body of the thesis, the symbol P and/or 0.05 indicate a statistically significant or non-significant differences respectively.

V- TISSUE PREPARATION

The experimental animals with their age-matched controls, were decapitated (small animal guillotine) two days after the last training session. Immediately after the sacrifice the thoracic cage was cut opened and the heart quickly removed, dissected out, rinsed in saline solution and wiped from excess water and weighed. The atria, valves, right ventricle, excess fat and the septum were then removed. The remaining free-wall of the left ventricle was weighed and frozen in liquid nitrogen and stored at -60^{0}C until ready for analysis.

VI- BIOCHEMICAL PROCEDURES

A- TISSUE HOMOGENATE

The frozen left ventricles were left standing at room temperature for about 15 minutes and then homogenized in a tight fitted Polytron homogenizer in re-distilled water (1 part in 20 volumes). Parts of the homogenate were used for DNA and RNA extractions (2.0 ml), protein (0.5 ml) and hydroxyproline determinations (1.0 ml). All the biochemical analyses were done in duplicate.

B- PROTEIN, NUCLEIC ACID AND HYDROXYPROLINE DETERMINATIONS

Duplicate protein determinations of 0.5 ml aliquots of the homogenates were performed using 4.0 ml of the biuret reagent to solubilize protein as outlined by Layne (1957). The purple coloured samples were read in a spectrophotometer at 550 nm following 30 minutes at room temperature (Appendix B-I).

The DNA and RNA were extracted with a modification of the Munro and Fleck's (1966) modification of the Schmidt-Thannhauser's method (1945). A 1.0 ml volume of 10% cold perchloric acid (PCA) and a 2.0 ml volume of methanol were added to 2.0 ml aliquots of the homogenate. The samples were centrifuged at 4000 x g for 20 minutes and the resulting precipitates were washed and recentrifuged twice in cold 0.2N PCA. To dissolve the precipitate 4.0 ml of 0.3N NaOH were added and the resulting alkaline solution was incubated for 1 hour in an incubator set at 37^{o}C. The alkaline digest

was then acidified by adding 2.0 ml of 1.2N PCA and the acid-soluble fraction obtained after centrifuging at 4000 x g for 20 minutes contained the RNA fraction. The precipitates were washed and recentrifuged twice in cold 0.2N PCA. The supernatant of these washings combined to the RNA fraction obtained from the acidification, contained the ribonucleotide products of RNA hydrolysis. To the remaining pellets, a 4.0 ml volume of 0.6N PCA was added and the DNA hydrolysis was obtained after heating in boiling water for 15 minutes. The DNA fraction was finally extracted from the supernatant after centrifuging at 4000 x g for 15 minutes.

Details of the nucleic acid extractions are outlined in Appendix B-II.

RNA was determined colorimetrically by the use of the orcinol reaction as suggested by Schneider (1957). The green coloured samples were then read at 660nm in a spectrophotometer (Appendix B-III).

DNA was also determined colorimetrically by the indole method of Ceriotti (1955) as modified by Keck (1956) by reading the yellow-brown coloured samples in a spectrophotometer at 490 nm (Appendix B-IV).

The method of Neuman and Logan (1950) was used for determining hydroxyproline. A 1.0 ml volume of concentrated hydrochloric acid (HCL) was added to 1.0 ml aliquots of the homogenate resulting in a final concentration of 6N HCL. The acid solution was autoclaved at 124^{o}C for 3 hours. After cooling for one hour the hydrolyzate was neutralized with

3N NaOH after adding three to four drops of Congo red indicator. The neutralized solution was then diluted to 10 ml with water, decolorized with charcoal (Norit A) and finally filtered after about 5 minutes. Details of the color reaction procedure for hydroxyproline measurement are outlined in Appendix B-V.

C- CALCULATION OF DIFFERENT PARAMETERS

1- NUMBER OF NUCLEI

The total number of nuclei which is a representation of cell number of the left ventricle was estimated by the following equation:

$$\text{Total number of nuclei}(10^6) = \frac{\text{total DNA of left ventricle(mg)} \times 10^3}{6.2}$$

where 6.2 is the amount of DNA in picograms (pgm) in a diploid nucleus (Enesco and Leblond, 1962; Winick and Noble, 1965).

2- WEIGHT PER NUCLEUS

The weight per nucleus which is a representation of the cell size of the left ventricle was determined by dividing total left ventricular weight by the number of nuclei as follows:

$$\text{Weight/nucleus(ngm)} = \frac{\text{total left ventricular weight(gm)} \times 10^3}{\text{total number of nuclei(millions)}}$$

3- ADJUSTMENTS OF HEART AND LEFT VENTRICULAR WEIGHTS

The weight of the heart and of the left ventricle from animals with different body weights may be adjusted to the body weight of reference animals according to Müller (1975) with the following equation:

$$g_{in} = g_i \cdot \left(\frac{G_c}{G_i}\right)$$

where g_i and G_i are the unadjusted means of muscle weight and body weight of the animals respectively; G_c is the mean body weight of the control groups (the reference group) and, g_{in} is the adjusted mean muscle weight.

RESULTS

The results of the dependent variables investigated in the present study are divided into five major sections as follows: 1- body, heart and left ventricular weights; 2- left ventricular protein content, concentration and protein to DNA ratio; 3- left ventricular RNA to DNA ratio, RNA content and concentration; 4- left ventricular DNA content and concentration, total number of nuclei and weight per nucleus; and 5- left ventricular hydroxyproline content and concentration. The raw scores and means and standard error of the means of the dependent variables as listed above, may be found in Appendix C. A code or symbol representing groups of animals with their age at the time of sacrifice will be used throughout the description of the results as previously described in Table 1. The summary of these symbols is: BC5: 5 week basic control group; C14: 14 week control group (age-matched with AN14 and A14); AN14: 14 week anaerobic trained group; A14: 14 week aerobic trained group; C25: 25 week control group (age-matched with AN25 and A25); AN25: 25 week anaerobic trained group; A25: 25 week aerobic trained group; C33: 33 week control group (age-matched with AN33 and A33); AN33: 33 week anaerobic trained group; A33: 33 week aerobic trained group.

1- BODY WEIGHTS, HEART WEIGHTS AND LEFT VENTRICULAR WEIGHTS

Figures 1.1 to 1.5 represent the body weight, the absolute and adjusted heart and left ventricular weights of

all groups of animals studied.

The mean body weight of the BC5 was significantly lighter than all the other control and trained groups (Figure 1.1). Also, all of the 14-week groups (C14, AN14 and A14) were significantly lower in body weight than the 25-week (C25, AN25 and A25) and 33-week (C33, AN33 and A33) groups. No significant differences were observed in the body weights of the 25 and 33 week groups. The aerobic training program significantly decreased the body weights of A14 and A25 when compared to their age-matched control groups respectively. The AN25 had a heavier ($P < 0.05$) body weight than the A25. Even though the mean body weight of the A33 was lighter than C33 and the AN33, this difference was not significant.

The heart and left ventricular weights will be described in the following order: the effects of growth on absolute heart and left ventricular weights, and on adjusted heart and left ventricular weights followed by the effects of the training programs on absolute and adjusted heart and left ventricular weights.

Lower mean differences in the absolute heart and left ventricular weights of the BC5 were significantly different as compared to control and trained groups (Figure 1.2 and 1.4). Differences ($P < 0.05$) were also observed in the absolute heart weights of the C14 as compared to the C25. The absolute heart weights of the AN14 was significantly lighter than the AN25 and AN33. Similarly the A14 had a significantly lower absolute heart weight than the A25 and

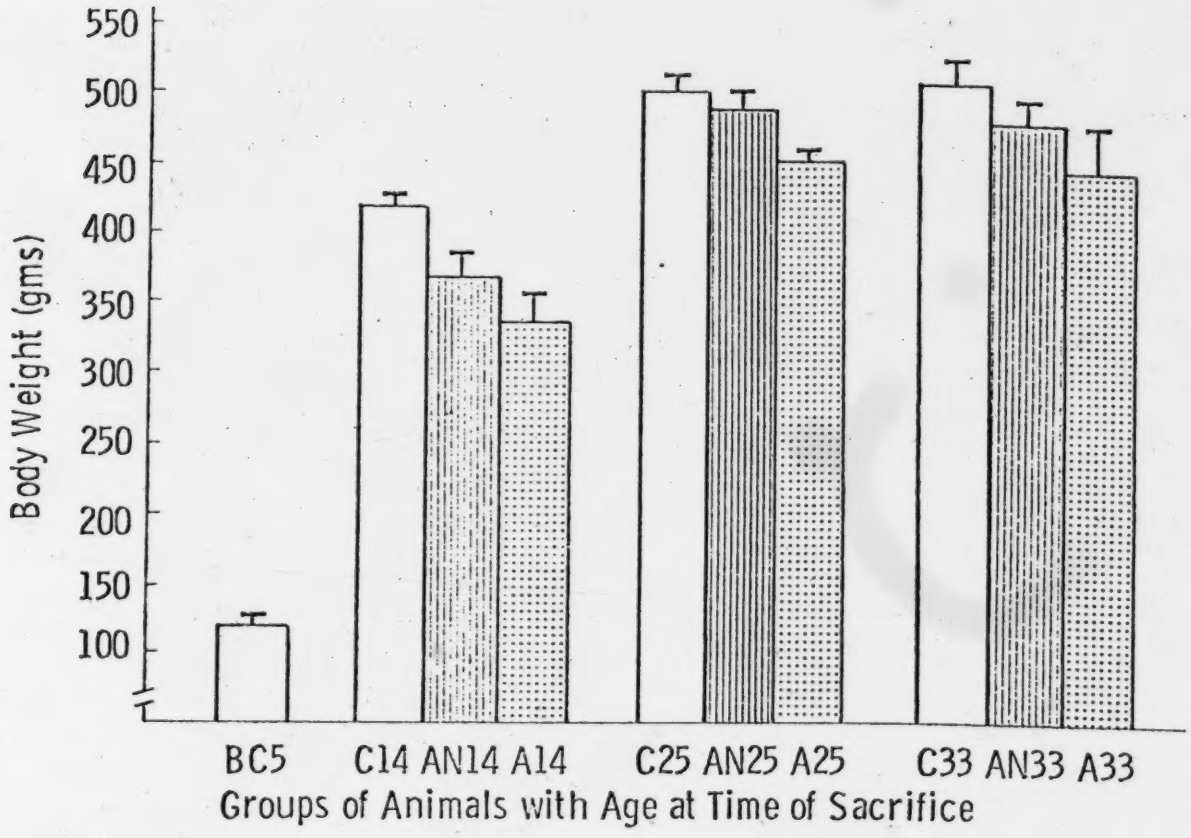

Figure 1.1 - The Effects of Growth, Anaerobic and Aerobic Training on Body Weight.

BC5 significantly lighter than all groups
C14 significantly lighter than C25 and C33
AN14 significantly lighter than AN25 and AN33
A14 significantly lighter than A25 and A33
A14 significantly lighter than C14
A25 significantly lighter than C25 and AN25

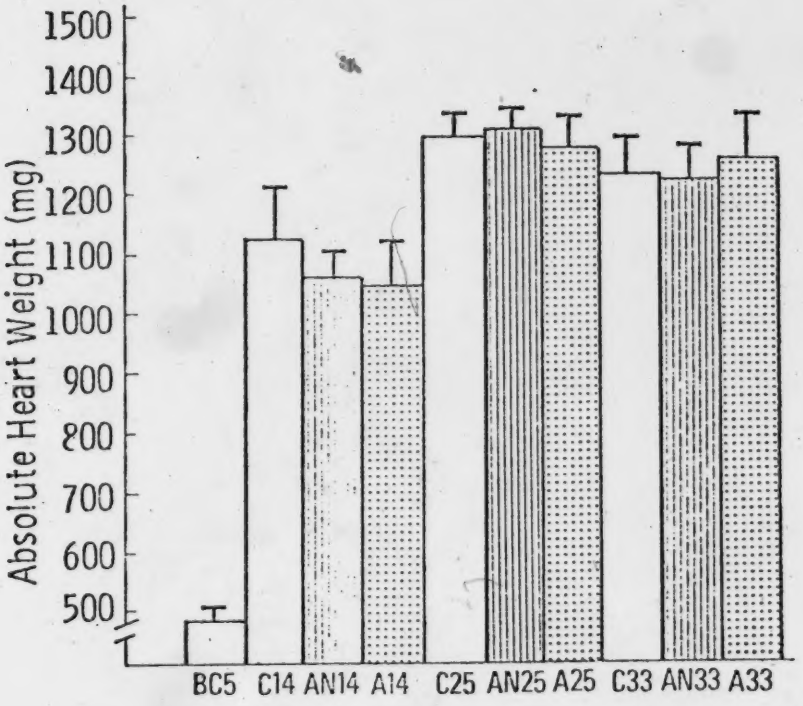

Figure 1.2 - The Effects of Growth, Anaerobic, and Aerobic Training on Absolute Heart Weight.

BC5 significantly lighter than all groups
C14 significantly lighter than C25
AN14 significantly lighter than AN25 and AN33
A14 significantly lighter than A25 and A33

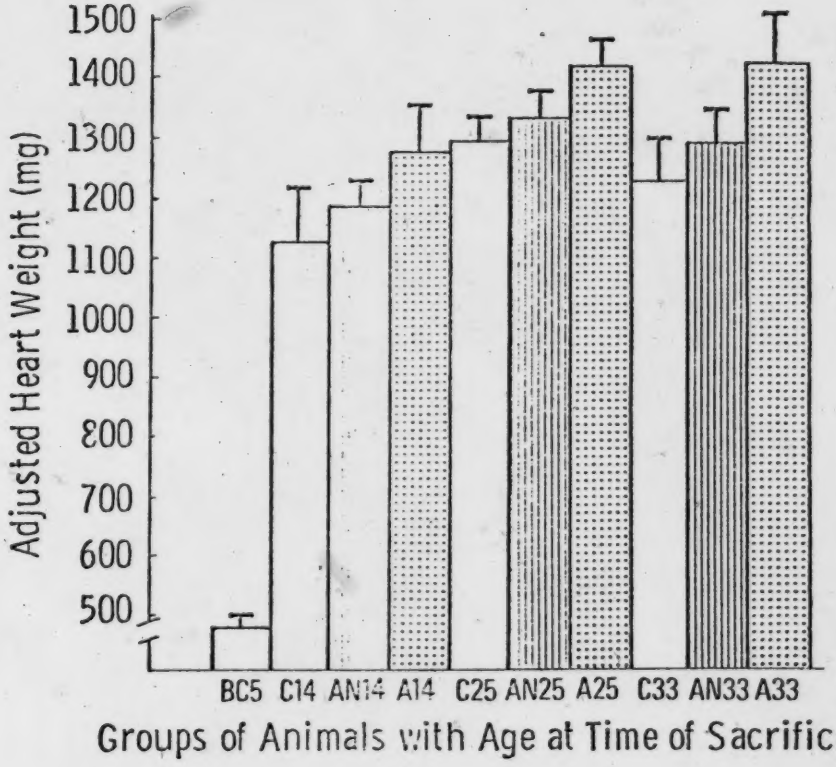

Figure 1.3 - The Effects of Growth, Anaerobic and Aerobic Training on Adjusted Heart Weight

BC5 significantly lighter than all groups
C14 significantly lighter than C25 and C33
AN14 significantly lighter than AN25
C25 significantly lighter than A25

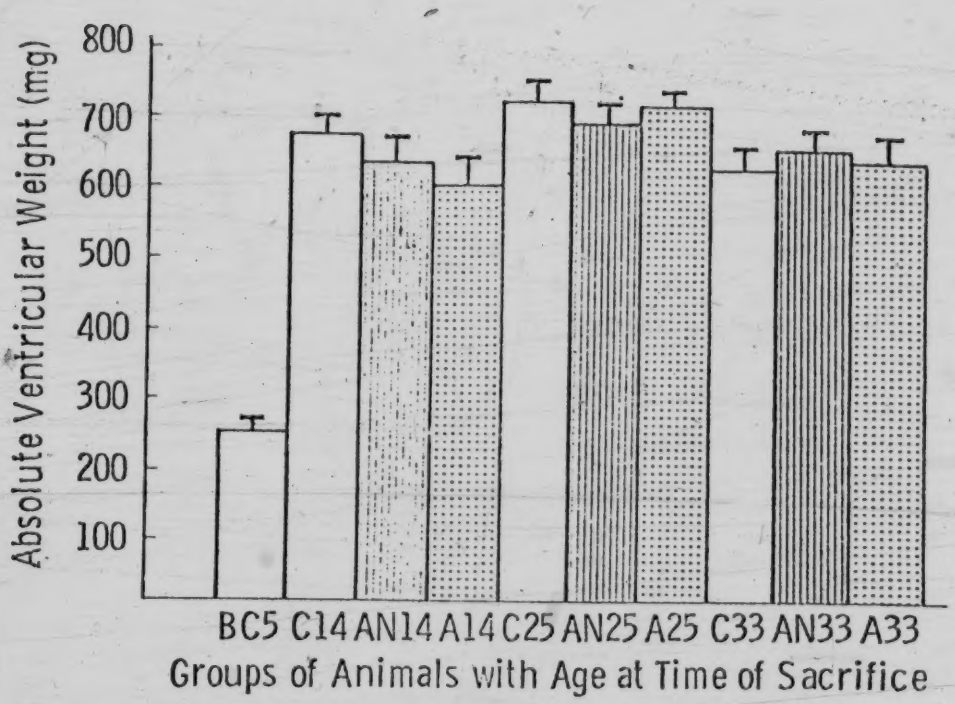

Figure 1.4 - The Effects of Growth, Anaerobic and Aerobic Training on Absolute Left Ventricular Weight.

BC5 significantly lighter than all groups
C33 significantly lighter than C25
A14 significantly lighter than A25

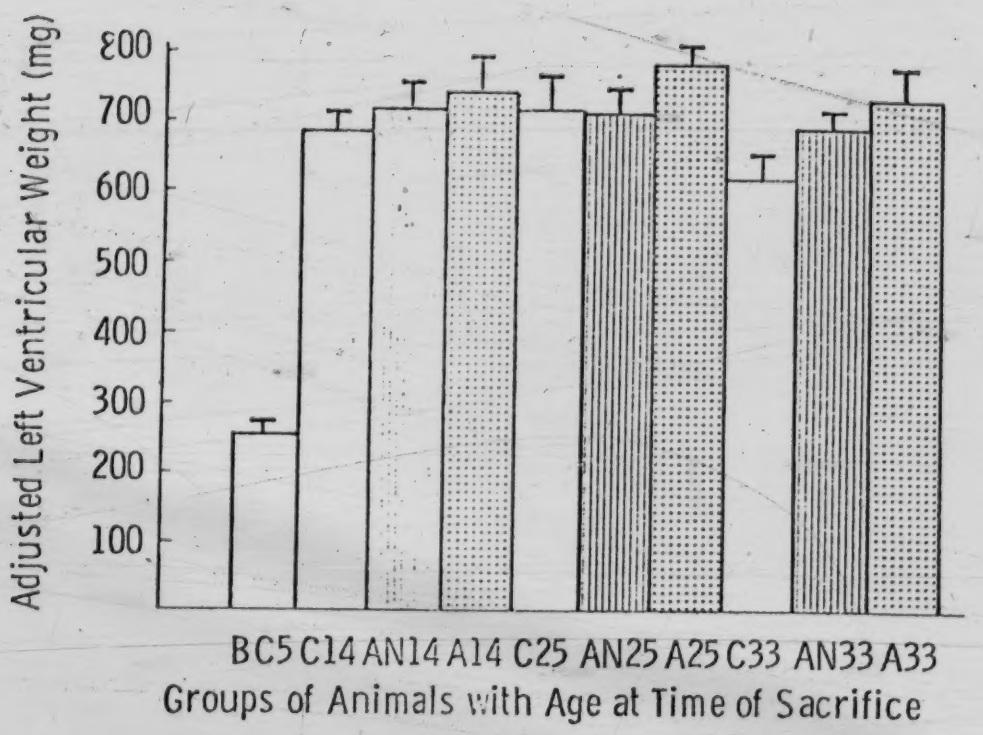

Figure 1.5 - The Effects of Growth, Anaerobic and Aerobic Training on Adjusted Left Ventricular Weight

BC5 significantly lighter than all groups
C33 significantly lighter than C25
C33 significantly lighter than AN33 and A33

A33.

The absolute left ventricular weight of the C33 was significantly lighter than the C25. Significant mean differences were also observed in the absolute left ventricular weights of the A14 when compared to the A25.

Heart weights and left ventricular weights were adjusted to account for differences in body weights of the trained animals at the time of sacrifice according to Müller (1975). The adjusted heart weight of the AN14 was found to be significantly lower than AN25 (Figure 1.3). No significant mean differences were obtained in the adjusted left ventricular weights of the aerobically trained groups (Figure 1.5).

The training programs did not produce significant differences between absolute heart and left ventricular weights. However, the aerobic training significantly produced a larger adjusted heart weight of the A25 when compared to its age-matched control, C25. Both training programs maintained a larger left ventricular weight in the AN33 and A33 as compared to C33. No interaction effects were observed in the three weights parameters studied.

Complete body, heart and left ventricular weight data are outlined in Appendix C-I.

2- LEFT VENTRICULAR PROTEIN CONTENT, CONCENTRATION AND PROTEIN TO DNA RATIO

Figures 2.1 to 2.3 represent the mean values of the protein content, concentration and protein to DNA ratio of the animals under investigation.

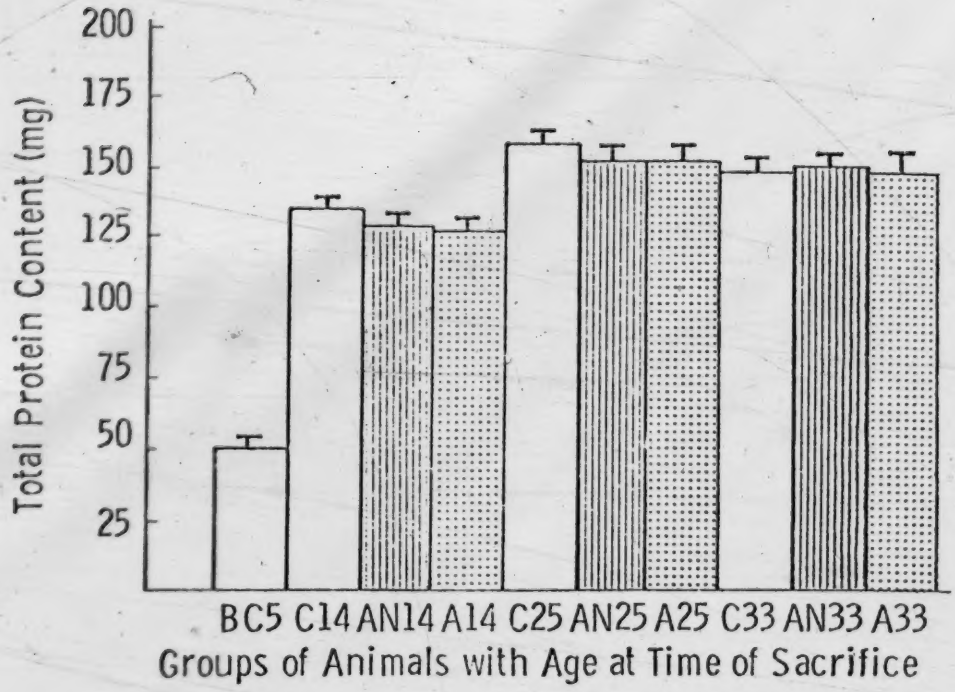

Figure 2.1 - The Effects of Growth, Anaerobic and Aerobic Training on Total Protein Content.

BC5 significantly lower than all groups
C14 significantly lower than C25

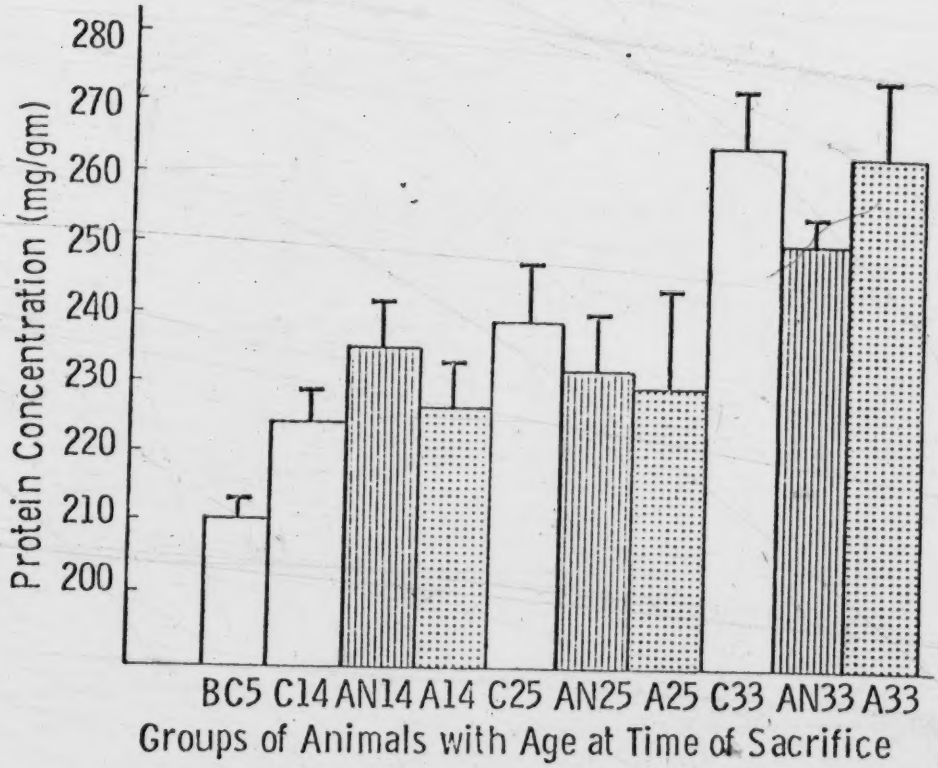

Figure 2. 2 - The Effects of Growth, Anaerobic and Aerobic Training on Protein Concentration.

BC5 significantly lower than C25, C33, AN14, AN25, AN33 and A33

C14 and C25 significantly lower than C33

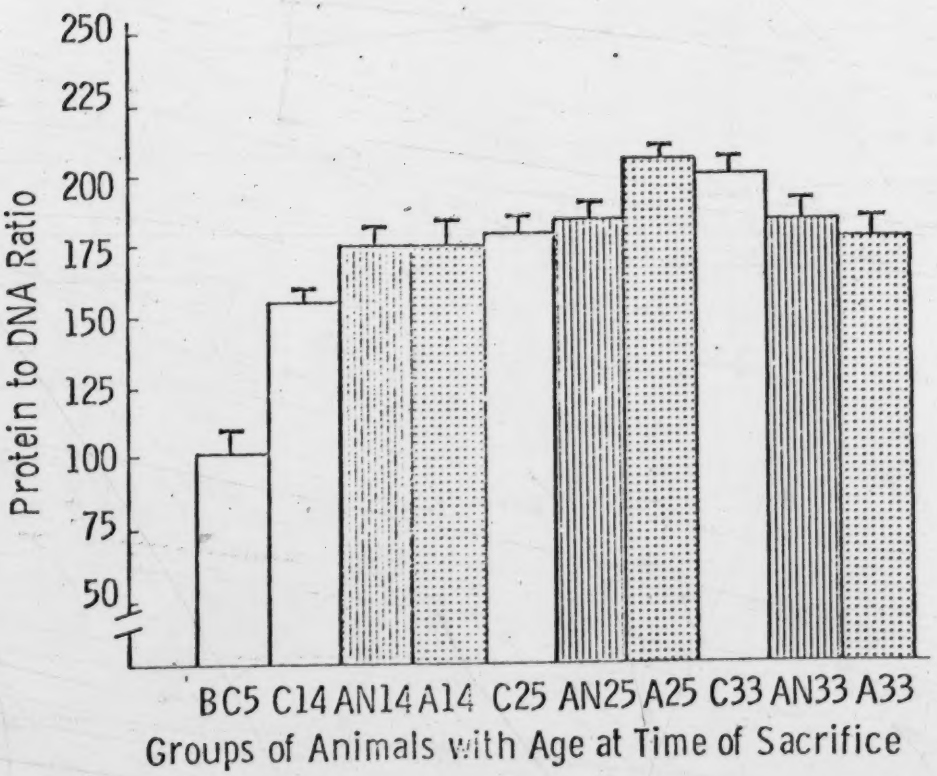

Figure 2.3 - The Effects of Growth, Anaerobic and Aerobic Training on Protein to DNA Ratio.

BC5 significantly lower than all groups
C14 significantly lower than C33

The protein content in the left ventricles of the BC5 were significantly lower than all groups of animals studied (Figure 2.1). The mean differences of the C25 were significantly higher than the C14. Neither training programs produced changes in the protein content of the trained animals.

Protein concentration of the BC5 was not significantly different when compared to C14, A14 and A25, but was significantly less concentrated than the remaining groups of animals. (Figure 2.2). The mean protein concentration of the C33 was significantly higher than the value of C14 and C25. Although trained animals in the 33-week aerobic and anaerobic groups were 33 and 19 mg/gm of wet weight higher than the 25-week trained groups respectively, these differences were not significant.

Significant growth differences in the protein to DNA ratio were observed in the BC5 as compared to all groups of animals (Figure 2.3). The protein to DNA ratio of the C33 increased significantly above the 14-week control group. The anaerobic and aerobic training programs did not produce significant alterations in the protein per DNA ratio.

The statistical analysis did not reveal interaction effects in the above parameters.

Total left ventricular protein, concentration and protein to DNA ratio data may be found in Appendix C-II.

3- LEFT VENTRICULAR RNA TO DNA RATIO, RNA CONTENT AND CONCENTRATION

Figures 3.1 to 3.3 represent the results of the

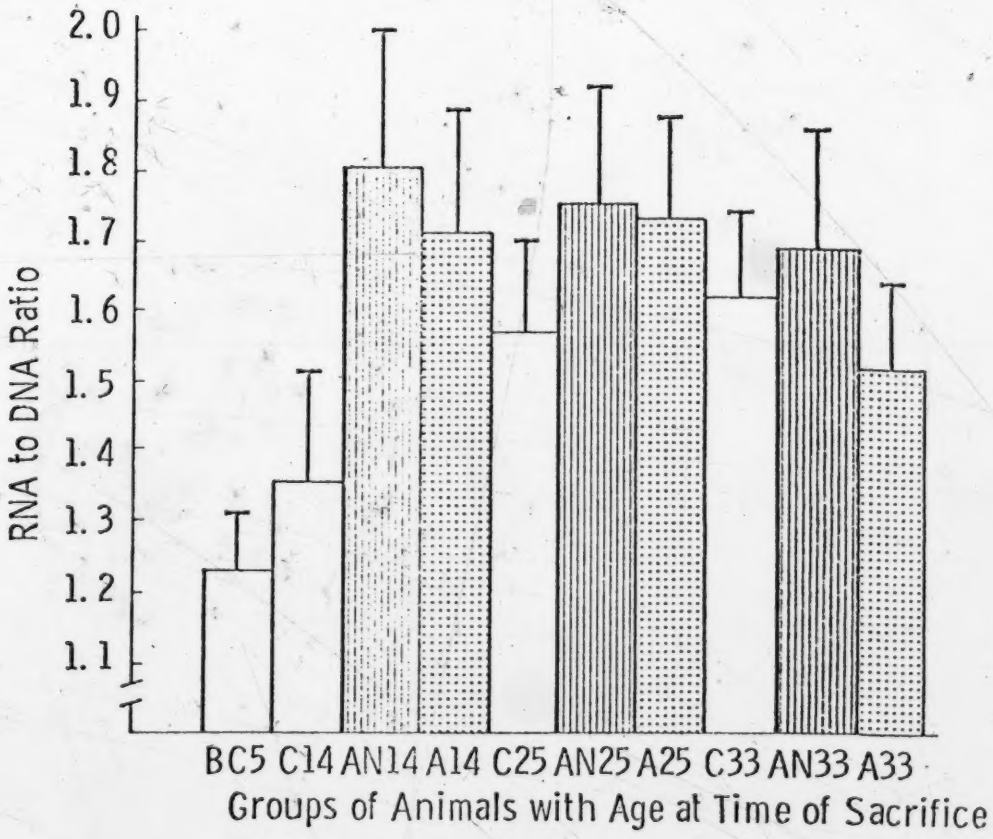

Figure 3.1 - The Effects of Growth, Anaerobic and Aerobic Training on RNA to DNA Ratio.

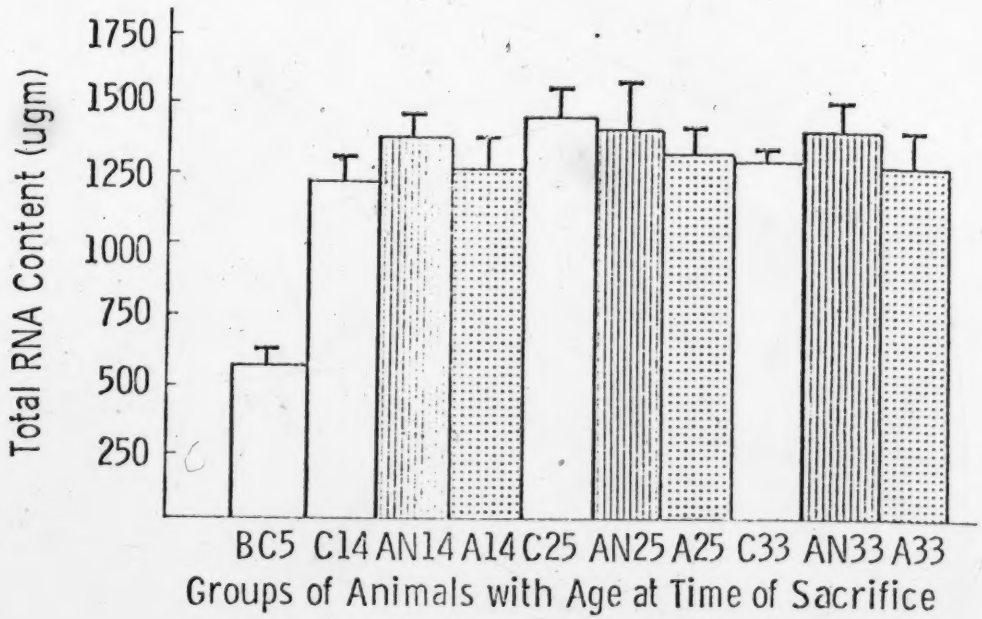

Figure 3.2 - The Effects of Growth, Anaerobic and Aerobic Training on Total RNA Content.

BC5 significantly lower than all groups.

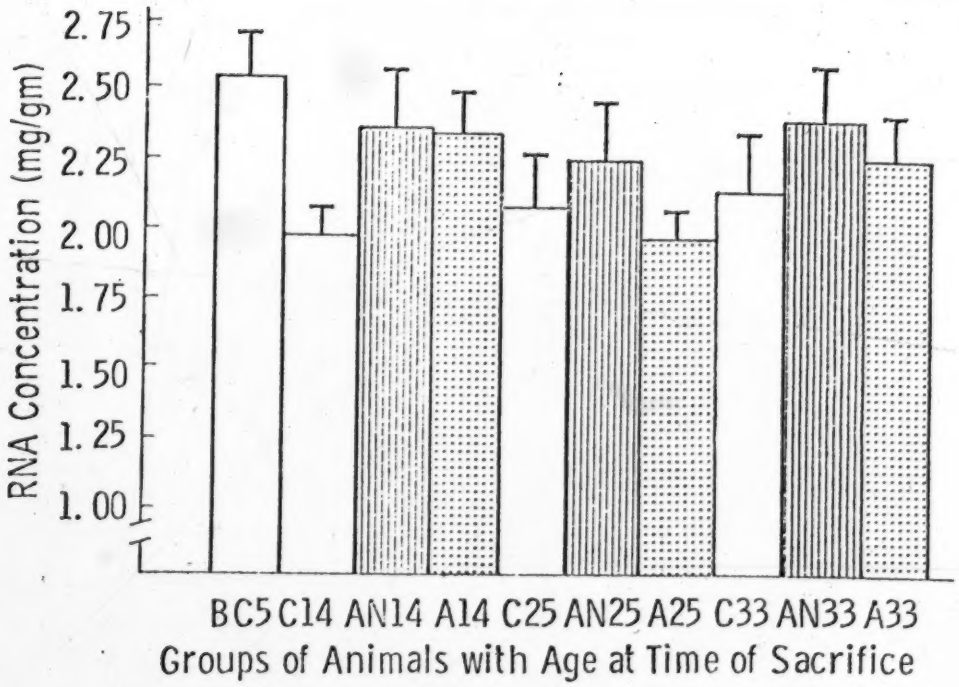

Figure 3.3 - The Effects of Growth, Anaerobic and Aerobic Training on RNA Concentration.

RNA to DNA ratio, RNA content and concentration of all the animals under study.

No significant mean differences were observed in the RNA to DNA ratio in all the animals and between control and trained animals (Figure 3.1).

Only the BC5 that showed a significantly lower RNA content when compared to all groups of animals (Figure 3.2). The same group of animals were not significantly different in the RNA concentration (Figure 3.3) from all the groups of animals. Training did not alter the growth pattern of the RNA content and concentration. Also, no interaction effects were found in the above parameters.

Complete RNA to DNA ratio, RNA content and concentration data are outlined in Appendix C-III.

4- LEFT VENTRICULAR DNA CONTENT AND CONCENTRATION, TOTAL NUMBER OF NUCLEI AND WEIGHT PER NUCLEUS

Figures 4.1 to 4.4 represent the mean values for DNA content and concentration, total number of nuclei and weight per nucleus for all the animals investigated.

Significant growth changes in the total DNA occurred in the left ventricles of the BC5 when compared to the 14, 25 and 33-week control and trained groups (Figure 4.1). Training did not produce changes in the DNA content of either training groups over their age-matched controls.

The DNA concentration of the BC5 was significantly higher than the control and trained animals (Figure 4.2). The A25 showed a significant decrease in the DNA concentration

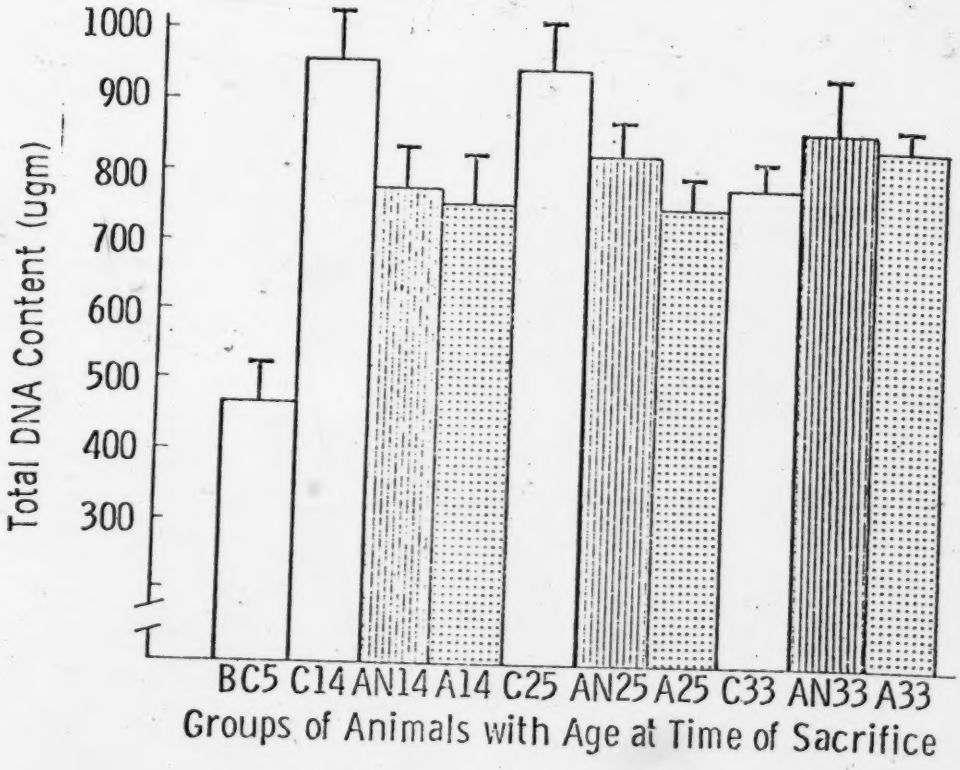

Figure 4.1 - The Effects of Growth, Anaerobic and Aerobic Training on Total DNA Content.

BC5 significantly lower than all groups

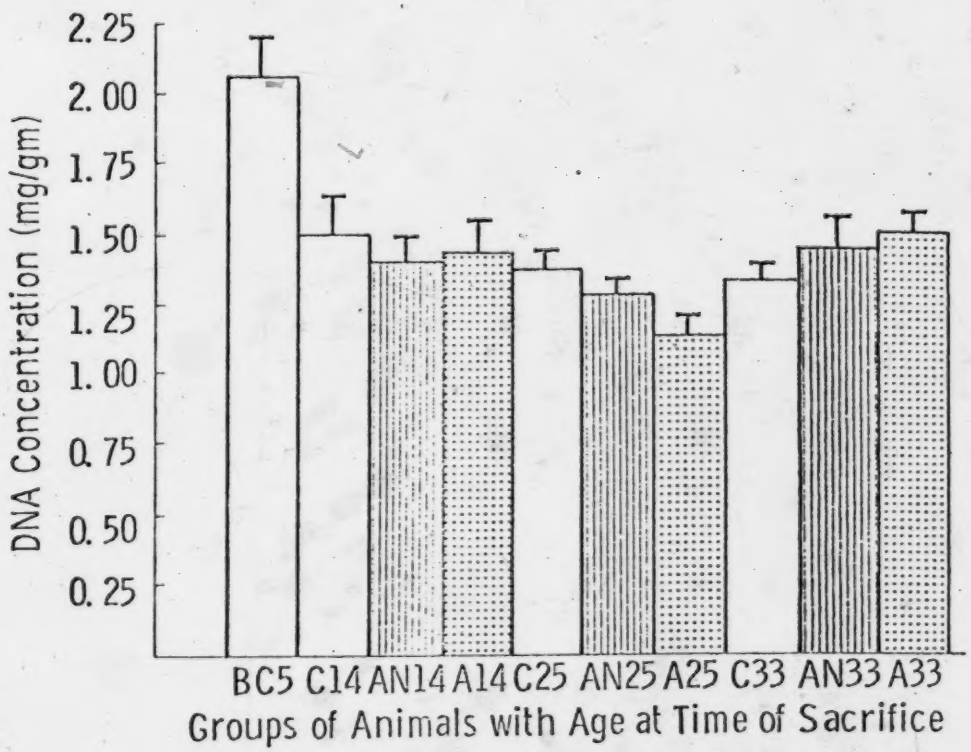

Figure 4.2 - The Effects of Growth, Anaerobic and Aerobic Training on DNA Concentration

BC5 significantly lower than all groups
A25 significantly lower than C25

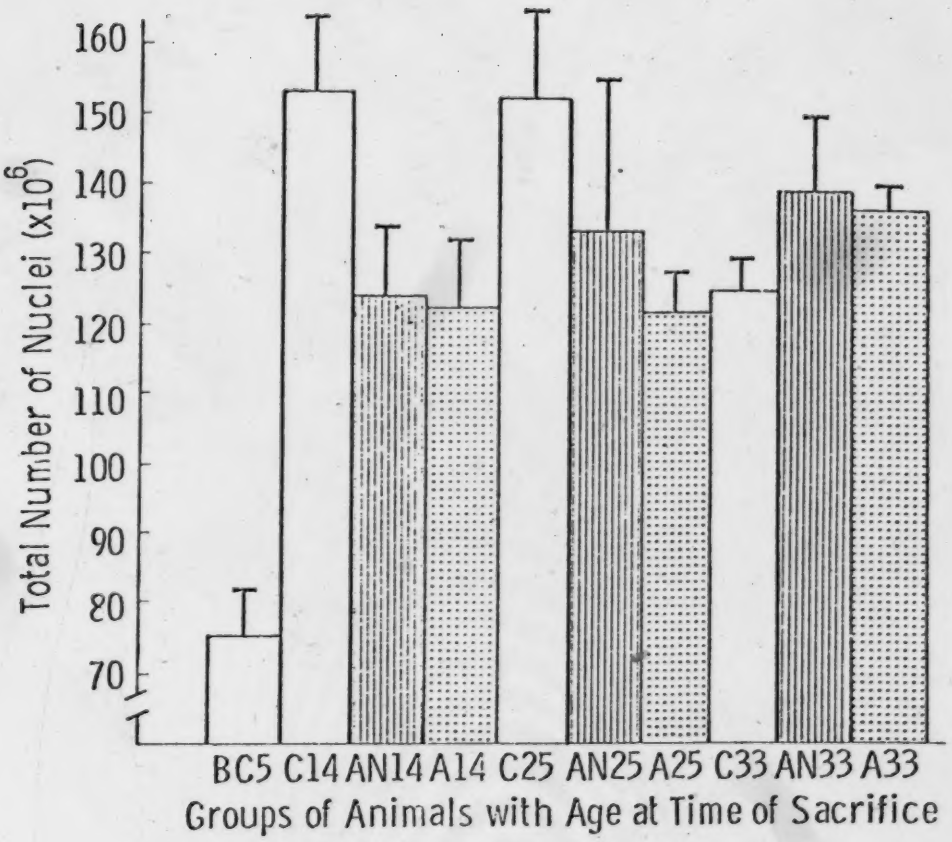

Figure 4.3 - The Effects of Growth, Anaerobic and Aerobic Training on Total Number of Nuclei.

BC5 significantly lower than all groups

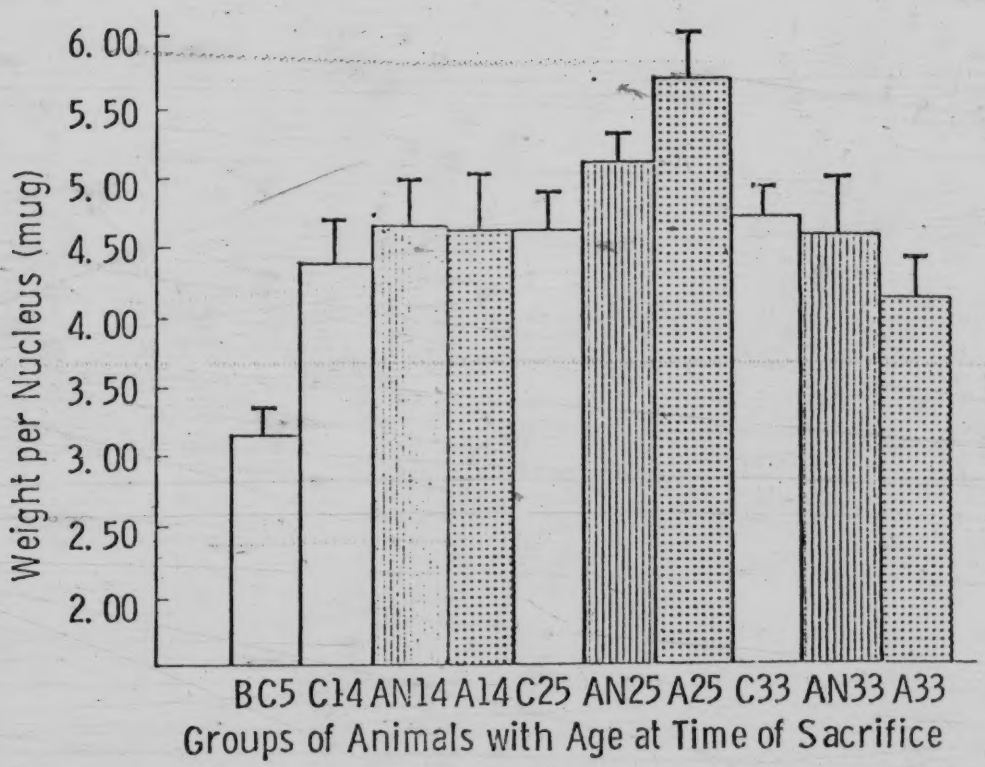

Figure 4.4 - The Effects of Growth, Anaerobic and Aerobic Training on Weight per Nucleus.

BC5 significantly lighter than all groups
A14 and A33 significantly lighter than A25
C25 significantly lighter than A25

over time of training, when compared to the A14 and A33 groups. The endurance training elicited a significant decrease in the DNA concentration of the A25 when compared to its age-matched control, C25.

Significant growth changes in the total number of nuclei and weight per nucleus were observed in the BC5 over all control and trained animals (Figure 4.3 and 4.4). The A25 demonstrated a significant increase in the weight per nucleus as compared to the A14 and to the A33. The aerobic training program increased significantly the weight per nucleus of the A25 over its age-matched control, C25. In contrast, both training programs did not alter the total number of nuclei in the left ventricle. No interaction effects were noticed in the above variables.

Data pertaining to the DNA content and concentration, to the total number of nuclei may be located in Appendix C-IV and the data weight per nucleus results in Appendix C-V..

5- LEFT VENTRICULAR HYDROXYPROLINE CONTENT AND CONCENTRATION

Figures 5.1 to 5.2 represent the results for hydroxyproline content and concentration in the left ventricles for all groups of animals investigated.

Left ventricular hydroxyproline content of the BC5 was significantly lower than the animals in the 14, 25 and 33-week control and trained groups (Figure 5.1). The AN33 had significantly more total hydroxyproline than did the AN14 and the AN25, while the A14 had significantly less than

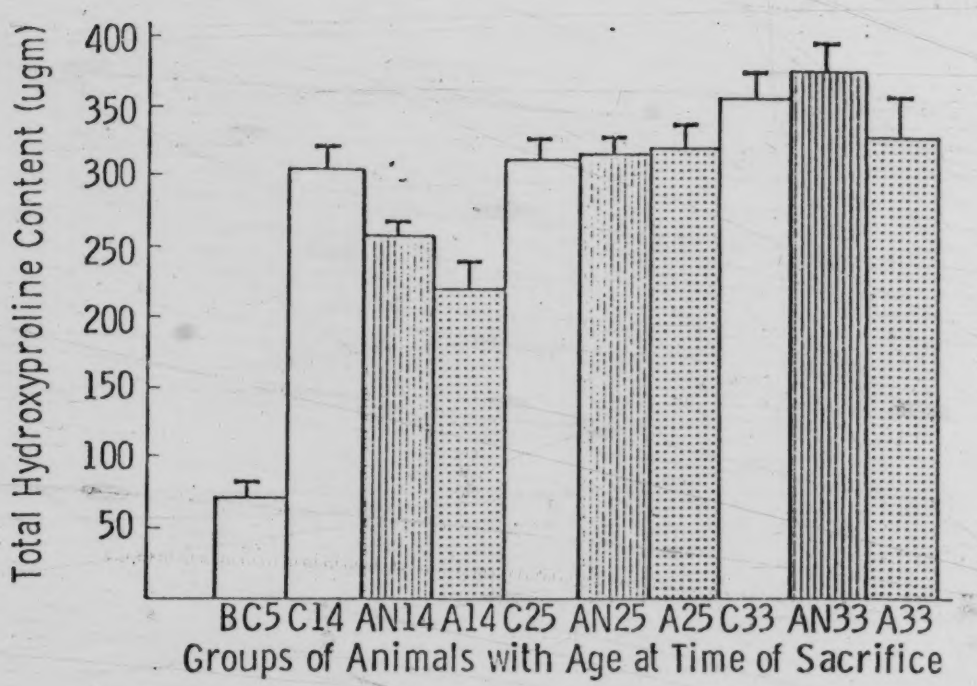

Figure 5.1 - The Effects of Growth, Anaerobic and Aerobic Training on Total Hydroxyproline Content.

BC5 significantly lower than all groups
AN14 significantly lower than AN25 and AN33
AN25 significantly lower than AN33
A14 significantly lower than A25 and A33
A14 significantly lower than C14

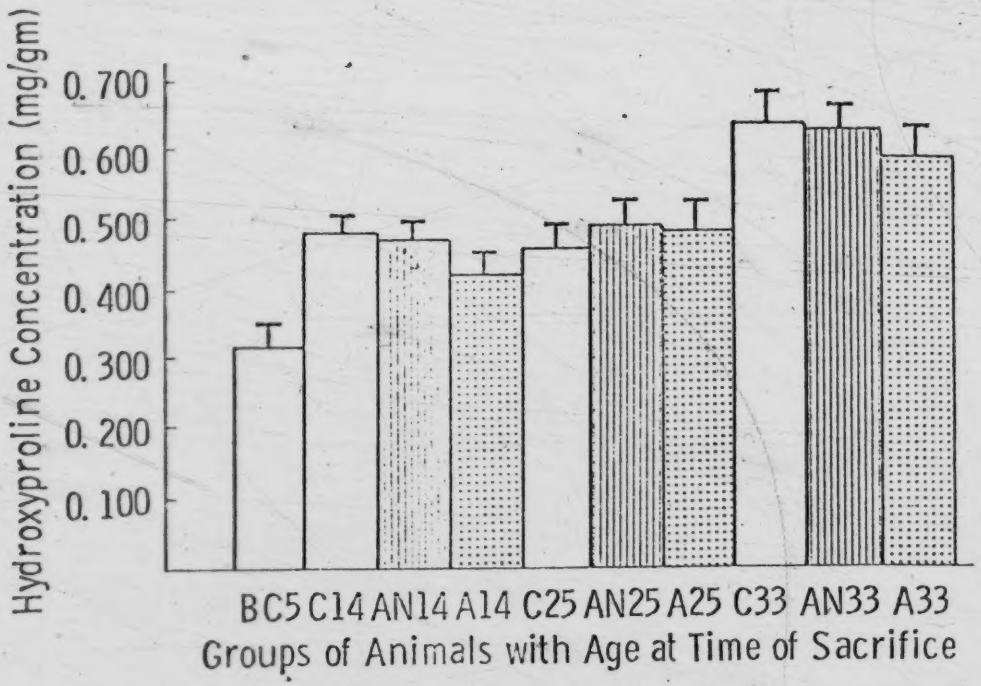

Figure 5. 2 - The Effects of Growth, Anaerobic and Aerobic Training on Hydroxyproline Concentration.

BC5 significantly lower than all groups
C14 and C25 significantly lower than C33
AN14 and AN25 significantly lower than AN33

the A25 and the A33 groups. Overall, the A33 and AN33 groups had significantly more total hydroxyproline in the left ventricles than the A14 and AN14 groups respectively. The aerobic training decreased significantly the total content of hydroxyproline of the A14 when compared to its age-matched control, C14.

While the hydroxyproline concentration of the BC5 was significantly less than the 14, 25 and 33-week control and trained animals, the C33, A33 and AN33 groups were significantly more concentrated than the 14 and 25-week control, aerobic and anaerobic groups respectively (Figure 5.2). No interaction effects were observed in hydroxyproline content and concentration.

Appendix C-V contained the data for left ventricular hydroxyproline content and concentration.

DISCUSSION

The discussion of the dependent variables is presented under five major sections as mention previously: 1- body, heart and left ventricular weights; 2- left ventricular protein content, concentration and protein to DNA ratio; 3- left ventricular RNA to DNA ratio, RNA content and concentration; 4- left ventricular DNA content and concentration, total number of nuclei and weight per nucleus; and 5- left ventricular hydroxyproline content and concentration. A code or symbol representing groups of animals with their age at the time of sacrifice will be used throughout the discussion as previously described in Table I.

The purpose of the present study was an attempt to differentiate the effects of anaerobic and aerobic training programs extending over several months on selected structural and biochemical parameters in the left ventricle of male rats.

1- BODY, HEART AND LEFT VENTRICULAR WEIGHTS

The body weights of the growing animals increased continuously up to 25 weeks of age and then plateaued with advanced aging. (Figure 1.1). Other studies substantiated this finding (Bloor and Leon, 1970; Oscai et al., 1971 a; Jaweed et al., 1974; Houston and Green, 1975; Pitts and Bull, 1977). According to Oscai et al. (1971 a), the effect of an endurance treadmill running program tended to produce a levelling off of the body weight between 340 and 410 gms in

male rats on a diet of Purina Chow. In the same study, the control rats on the same diet continued to gain weight with age until they reached about 650 to 700 gms. Consequently, the control animals actually became obese throughout their sedentary life span. However, the animals subjected to aerobic training did not gain as rapidly in body weight following 9 and 20 weeks of training when compared to their age-matched controls. Similar findings concerning a reduction in body weight have been reported as a result of endurance training (Bloor and Leon, 1968; Crews et al., 1969; Bloor et al., 1970; Penpargkul and Scheuer, 1970; Oscai et al., 1971 a; Houston and Green, 1975; Dowell et al., 1976 a; Codini et al., 1977).

Although the anaerobically trained animals were not significantly different in body weight than age-matched controls and aerobic trained animals, the weights tended to be somewhat lower than the age-matched controls and heavier than the aerobically trained animals respectively. Non-significant reductions in the body weight have been reported in anaerobic types of training programs (Exner et al., 1973; Jobin, 1977). Contrary to these findings, other researchers reported that high intensity training decreased the body weight of male rats (Staudte et al., 1973; Houston and Green, 1975; Hickson et al., 1976). It was suggested by Oscai et al. (1971 a) that the reduction in the body weight of trained animals was the result of an increase in caloric expenditure and a decrease in the caloric intake. This appetite-suppres-

sing effect was thought to be related to the amount of stress evoked by the work load placed on the animals rather than the amount of exercise per se (Crews et al., 1969). This seemed to be the case only in older animals (Bloor and Leon, 1970) while younger animals tended to be influenced by the amount of exercise performed (Bloor and Leon, 1970; Oscai et al., 1971 a; Dowell et al., 1976 a). Furthermore, the reduction in body weight of trained animals could be accounted for on the basis of the lower fat content observed after training (Crews et al., 1969). According to Parizkova and Stankova (1967) and Parizkova and Koutecky (1968), trained animals were usually characterized by an increase in the mobilization of FFA from the adipose tissu, a decreased proportion of body fat and an increase lipo-protein lipase activity in the heart and skeletal muscles.

It seemed that the body weights of female rats tended to be less affected by both aerobic training programs (Crews and Aldinger, 1967; Arcos et al., 1968; Dowell et al., 1976 b) and anaerobic training programs (Jaweed et al., 1974; Baldwin et al., 1977). According to Dowell et al. (1976 b), female rats are not willing or able to perform treadmill exercises of the same intensity and duration as male rats. Researchers are now favouring the use of female rats to overcome the problem of comparing animals of different body weights as demonstrated frequently in male rats.

Findings from the present study seemed to substantiate that endurance rather than high intensity training

influenced the body weight to a greater degree. Since only aerobic training in the present study produced significantly lower body weights, this suggested that the amount of exercise, and not the increased stress placed on the animals was the predominant factor affecting the body weight. The anaerobic animals ran 20% of the total distance covered by the aerobic animals, showing further the importance of the amount of exercise performed (Appendix A-IV, Tables 3 and 4). Furthermore, the amount of exercise performed by the aerobically trained animals would seem to suggest a greater total energy expenditure, when compared to the anaerobically trained animals.

The absolute heart weight of the control animals increased significantly up to the age of 25 weeks and decreased slightly from 25 to 33 weeks (Figure 1.2). This finding is in agreement with the growth study of Jaweed et al. (1974) who reported a significant growth change up to 22 weeks of age. Thereafter, the heart weight plateaued with advanced aging. In the present study, the alteration in heart weight of growing control animals represented a threefold increase during the pre-pubertal period. This is substantiated by the works of Bell et al. (1975) and Winick and Noble (1965). These researchers noted a fourfold increase in heart weight in young control animals over the same age span as the present study. The absolute heart weight of the trained animals in this study followed a similar developmental pattern.

The growth in the heart seemed to be a result of

an increase in cell size (Rakusan, 1965; Zak, 1973), coincident with the cessation of the mitotic activity observed 3 to 8 weeks post-natally (Goss, 1964; Winick and Noble, 1965; Ostadal et al., 1967; Brown, 1971, Zak, 1973). This cessation of mitotic division in the developing heart paralleled by a decrease in cytoplasmic DNA polymerase, was thought to be the mechanism regulating mitotic activity in the heart (Doyle et al., 1974; Claycomb, 1975).

A similar growth pattern to 25 weeks of age (P 0.05) as shown with absolute heart weight was observed in the absolute left ventricular weight of the young control animals (Figure 1.4). Thereafter, it decreased significantly up to 33 weeks of age. The magnitude of change in the absolute left ventricular weight was greater (P 0.05) in pre-pubertal animals with aerobic training. Whereas in animals trained anaerobically, the absolute left ventricular weight was increased (P 0.05) up to 25 weeks of age and then somewhat decreased (P 0.05) in older animals.

Absolute parameters, such as the heart and left ventricular weights as mentioned above, are somewhat related to the size of the animals (Müller, 1975). As training influenced body weight as well as cardiac and/or skeletal muscle, these alterations in absolute tissue weight could be related to the changes in body weights and not necessarily attributable to the different treatments. To overcome the problem of comparing animals of different body weights, different procedures have been derived (Pattengale and Holloszy, 1967; Tomanek and Woo, 1970; Maher et al., 1972;

Exner et al., 1973). The procedure adopted in the present study to correct for the decrease in body weights of the trained animals was a modification of the method described by Müller (1975). Since the heart weight (Dunn et al., 1947; Vanliere and Northup, 1957; Grande and Taylor, 1965; Oscai et al., 1971 a) and left ventricular weight (Dowell et al., 1976 a) showed a parallel relationship with body weight in normal rats, the mean body weight of the control animals was used to adjust heart and left ventricular weights for differences in body weight. The adjusted heart and left ventricular weights are shown in Figures 1.3 and 1.5. The AN14 showed a significantly lower adjusted heart weight as compared to the AN25. Therefore, 20 weeks of anaerobic training increased significantly the absolute and adjusted heart weight of AN25. The difference was partially a result of a heavier absolute body weight in the AN25 in addition to a significantly lighter absolute heart weight of the AN14. This seemed to suggest that 20 weeks of training was of a sufficient overload that the heart adapted by increasing its mass and that further training did not stress the heart above the attained structural level.

Aerobic training seemed to induce cardiac hypertrophy following 20 weeks of training as shown in the significant increase in the adjusted heart weight in the A25 (Figure 1.3). Further evidence that cardiac enlargement occurred with endurance training in male rats has been well documented by other workers (Bloor et al., 1968; Bloor

and Leon, 1970; Oscai et al., 1971 a; Steil et al., 1975) and also in female rats (Crews and Aldinger, 1967; Vanliere et al., 1965; Arcos et al., 1968; Oscai et al., 1971 b; Jaweed et al., 1974). However, some studies have reported no cardiac hypertrophy due to aerobic training (Houston and Green, 1975; Dowell et al., 1976 b; Baldwin et al., 1977; Codini et al., 1977). Jaweed et al. (1974) suggested the possibility that high intensity training might produce cardiac enlargement as well as skeletal muscle hypertrophy. This hypothesis seemed to be refuted by the findings of the present study. Even though the anaerobic group showed heavier adjusted heart weights (P 0.05) than the control animals, this difference was not significant. That is, the anaerobic training program did not elicit an increase in the size of the heart. In regard to the skeletal muscle size, Wilkinson (1977) who used the same training program, showed that the skeletal muscle weights were also not affected by this anaerobic training regimen.

Since cardiac hypertrophy in aerobically trained animals seemed to have occurred in this study it should have enhanced the functional capacities of these hearts. Beznak (1958) and Arcos et al. (1968) proposed a positive correlation between heart weight and maximum cardiac output in relation to cardiac hypertrophy. Hence, an increase in the heart weight in aerobically trained animals can thus be associated with an increase in the maximum capacity to deliver blood to the exercising muscles. The cardiac efficiency can be

greatly improved if no hypertrophy of the skeletal muscle existed. This seemed to be true following endurance training (Holloszy, 1967; Pattengale and Holloszy, 1967; Jaweed et al., 1974). Also associated with cardiac hypertrophy are increased cardiac contractility, cardiac reserve (Crews and Aldinger, 1967) and myofibrillar ATPase (Baldwin et al., 1977) which would enhance contractile potential of the heart. Aerobic training was found to improve cardiac performance by altering the intrinsic characteristics of the myocardium with a concomittant increase in its functional properties (Crews and Aldinger, 1967; Penpargkul and Scheuer, 1970; Ford, 1976; Codini et al., 1977).

Although the one-way ANOVA showed significant simple main effects (P 0.05) in the adjusted left ventricular weight of the 25 week groups the Newman-Keuls post hoc test did not reveal significant differences between the means. The adjusted left ventricular weight of the A25 was 9% heavier than the C25. This non-significant increase was associated with a decrease (P 0.05) in DNA concentration and an increase (P 0.05) in the weight per nucleus. These latter changes suggested, in fact, that an increase in cell size had taken place without left ventricular hypertrophy. Matsumoto and Krasnow (1968) however, have demonstrated that when the DNA concentration decreased in the ventricles with an increase in heart weight there existed an indication of increase in the size of the ventricles. According to Enesco and Leblond (1962), the weight per nucleus represented the cell size in

a single diploid nucleus of the rat's heart, further suggesting, from the present study, that an increment in this parameter would reflect an increase in cell size. Jaweed et al. (1974) suggested that cardiac enlargement was due to an increase in fiber size and not to an increase in the number of fibers. The present findings tend to agree with this suggestion and, in fact, the fibers did hypertrophy after 20 weeks of aerobic training. No changes were reported in the DNA content and total number of nuclei (representing cell number) after 20 weeks of aerobic training.

There existed significant differences in the adjusted left ventricular weights in the AN33 after 28 weeks of training when compared to the control animals. This difference would suggest that an enlargement of the left ventricles had occurred. It was interesting to note that the left ventricular weights decreased (P 0.05) in 33-week trained animals and decreased significantly in the 33-week control animals respectively. This marked decline in ventricular mass of the control animals over the 25 week control group caused the 33-week old animals to be significantly different from their age-matched control group. It would be reasonable to conclude that the difference observed was not due to the anaerobic and aerobic training programs but to a significant loss in the ventricular mass of the control animals 33 weeks of age. It appeared that in the 33 week-old animals, aging initiated a catabolic response with an actual loss of ventricular mass. Such a loss could be associated with a loss

of myocardial fibers (Kloor and Leon, 1970). It would seem that cardiac hypertrophy or left ventricular hypertrophy had not occurred after 28 weeks of anaerobic and aerobic training. Both training programs seemed to have been able to maintain the weights of the left ventricle to the existing levels.

2- LEFT VENTRICULAR PROTEIN TO DNA RATIO, PROTEIN CONTENT AND CONCENTRATION

A significant increase in the protein to DNA ratio from 5 to 14 weeks of age in control and trained animals was demonstrated in the present study. Thereafter, the rate of increase declined in mature animals (Figure 2.3). The non-significant increase in protein to DNA ratio observed from 14 to 25 weeks may be a result of a balance between the DNA synthesis and protein accumulation. However, the 33-week-old control animals showed a significant increase in protein per DNA as compared to the control animals 14 weeks of age. This could be due to a slight decrease in the DNA content (P 0.05) (Figure 4.1) rather than an increase in the protein content (P 0.05) (Figure 2.1) in these mature animals (Winick and Noble, 1965).

No alterations from the normal control pattern in the protein to DNA ratio were demonstrated following 9, 20 and 28 weeks of training. The protein to DNA remained relatively constant between the control and trained animals, further substantiating the lack of change in the protein and DNA contents in response to chronic exercise (Figure 4.1 and 2.1 respectively).

The protein content of the growing left ventricle increased significantly from 5 to 14 weeks of age (Figure 2.1). Similar findings were reported by Bell et al. (1975) in the myocardium of animals 3 to 9 weeks of age. According to Winick and Noble (1965) normal growth in young animals seemed to be associated with a sharp rise in total protein in the whole heart. The magnitude of the increase up to 14 weeks observed in the present study was the same as that reported by Winick and Noble (1965) and less than that of Bell et al. (1975) in 3 to 9 week-old animals. It was observed in the present study that the protein content rose significantly up to 25 weeks of age in the control animals with a slight decrease ($P > 0.05$) at 33 weeks. However, there was no significant increase up to 25 weeks of age in either training group. This seemed to imply that both training programs resulted in a decrement in the net protein synthesis of the left ventricle when compared to their age-matched controls. Furthermore, in both control and trained animals the protein content plateaued between 25 and 33 weeks. This was in agreement with Winick and Noble (1965) and Grimm et al. (1966) who found that the protein content of the whole heart and left ventricle remained relatively constant in mature animals respectively. This would tend to imply that the relatively constant amount of protein found in young animals was maintained with advanced aging and that the existing levels were sufficient to meet the overload demands of both training programs.

No alteration in the protein content of the left ventricle was demonstrated between trained and control animals. This result was expected in the trained animals as the training did not cause an increase in the absolute left ventricular weight. Findings from other studies have reported no change in protein content as a result of training (Steil et al., 1975; Oscai et al., 1971 a; b; Dowell et al., 1976 a; Medugorac, 1976; Sordahl et al., 1977). This might suggest that the training programs did not represent a sufficient overload to increase the protein content above normal levels, or that in fact, the existing levels were large enough to meet the overload demand.

A significant growth effect in protein concentration of the 5 week-old animals was demonstrated when compared to the 33 weeks control and trained animals. The control animals showed a significant increase (P 0.05) in the protein concentration during growth between 25 and 33 weeks of age (Figure 2.2). The overall change in protein concentration in these animals was an almost linear increase with age (5 to 33 weeks of age). According to Young (1970) the cardiac muscle tended to increase in protein concentration with growth. The trained animals also showed an increase (P 0.05) in the protein concentration between 25 and 33 week-old animals, however, the magnitude of change was less pronounced than their age-matched control groups. This was due to somewhat lower (P 0.05) absolute left ventricular weights observed in these trained animals (Figure 1.4).

No changes were found in the protein concentration in either trained group following 9, 20 and 28 weeks of training (Figure 2.2). This was similar to the findings of Sordahl et al. (1977) who observed no differences in dogs submitted to 10 weeks of exercise. Similar findings have also been reported by Medugorac (1976) and Dowell et al. (1976 a) in young rats subjected to 10 and 11 weeks of chronic exercise respectively. According to Medugorac (1976), the concentration of myofibrillar protein in young rats submitted to pre-pubertal endurance training, was slightly but constantly increased always at the expense of sarcoplasmic and stromal proteins. Although the protein concentration in these fractions were not measured in the present study, a slight increase ($P < 0.05$) over age-matched controls in the protein concentration was noticed in aerobic and anaerobic animals 14 weeks of age. Oscai et al. (1971 b) have also shown that the cardiac mitochondria expressed per gram of ventricular muscle or per milligram of myocardial protein, did not undergo an adaptive increase in concentration in response to endurance exercise. It was demonstrated that the respiratory enzyme levels, expressed per gram of muscle were fivefold in the heart as compared to skeletal muscle in the sedentary rat (Oscai et al., 1971 a). This further substantiated the lack of change observed between control and trained animals in the present study. This may suggest that the respiratory enzyme levels of the cardiac muscle of the 5 week-old control animals was sufficiently large to meet

the increased demands for energy production imposed by the aerobic training program.

3- LEFT VENTRICULAR RNA TO DNA RATIO, RNA CONTENT AND CONCENTRATION

The RNA to DNA or the RNA per nucleus of the left ventricle was not affected by growth in animals between 5 to 33 weeks of age (Figure 3.1). According to Winick and Noble (1965), the RNA per nucleus varied with individual organs and was not affected by age. They showed a high RNA to DNA ratio during the pre-pubertal period. It would seem that RNA obtained a final content per nucleus or per cell in early growth of the heart, and stayed elevated thereafter. This would also imply that the RNA was sufficient to sustain normal rates of protein synthesis during normal growth. Winick and Noble (1965) have also shown that the RNA to DNA was relatively high all through the pre-pubertal period in the whole heart, suggesting that the heart was rich in RNA in early growth period. It was also demonstrated that the RNA per nucleus was elevated even during rapid cell division, implying a high content of RNA per cell even when synthesis of DNA was highest (Winick and Noble, 1965).

No changes were found, in the present study, in the RNA to DNA ratio following 9, 20 and 28 weeks of training. This seemed to indicate that the RNA per nucleus was sufficient to maintain the protein synthetic activity at normal levels in the left ventricle even under physical stress.

Similarly no alteration in the RNA concentration

was observed in 5 to 33 week-old control and trained animals (Figure 3.3). Although the differences were not significant, there existed a tendency towards higher values in the 5 week-old animals, thereafter, the RNA concentration decreased somewhat with advanced aging. This developmental pattern substantiated the findings of other researchers that young animals tended to show higher RNA per gm of myocardium than older animals (Winick and Noble, 1965; Grimm et al., 1966). No changes were observed following anaerobic and aerobic training extended over several months. This was in agreement with other studies (Dowell et al., 1976 a, b; Sordahl et al., 1977).

The RNA content was significantly lower in the 5 week-old animals as compared to all the animals studied (Figure 3.2). After 14 weeks the RNA content stayed relatively constant. According to Winick and Noble, (1965) the amount of RNA seemed to be sufficient in the whole heart, to sustain normal rates of protein synthesis during normal growth.

Neither training program elicited changes as compared to the control animals in net RNA synthesis in the left ventricle. Other studies have supported this finding (Dowell et al., 1976 a, b; Bell et al., 1975; Sordahl et al., 1977). According to Bell et al., if expected changes are to occur in the myocardial RNA, the training stimulus must be applied for as long a period as possible before the onset of puberty. Since no significant changes were found in the RNA

content between control and trained animals, it was unlikely that the adaptive response observed after 20 weeks of aerobic training in the adjusted heart weight, represented an increase in a net myocardial protein synthetic activity above normal level.

4- LEFT VENTRICULAR DNA CONTENT AND CONCENTRATION, TOTAL NUMBER OF NUCLEI AND WEIGHT PER NUCLEUS

DNA changes in growing left ventricle were marked by a significant increase in total DNA and a significant decrease in the DNA concentration in animals 5 to 14 weeks of age (Figure 4.1 and 4.2 respectively). Other studies have supported these findings (Enesco and Leblond, 1962; Winick and Noble, 1965; Grimm et al., 1966; Bell et al., 1975). An increase in total DNA content seemed to parallel the increase ($P < 0.05$) in the left ventricular weight in animals of the same age. According to Sasaki et al. (1968 b), total DNA content increased to the same extent as the growth of the whole heart. This would imply that the DNA was being synthesized in the left ventricle of young animals in this study. The present investigation also showed a significant increase in the total number of nuclei and weight per nucleus in the animals 5 to 14 weeks of age. Winick and Noble (1965) and Enesco and Leblond (1962), also showed an increase in these two parameters in the whole heart in 65 and 95 day-old animals respectively. This would suggest that during this developmental period, the left ventricular fibers were increasing both in number and in cell size.

The DNA concentration was significantly elevated in the 5 week-old animals when compared to all animals studied (Figure 4.2). According to Sasaki et al. (1968 b), the high DNA concentration observed in young animals was due to the fact that the cardiac muscle cells were smaller in size and grouped more compactly during growth period.

In animals 14 weeks to 33 weeks of age, the DNA content and total number of nuclei levelled off somewhat (P 0.05). This would suggest that the DNA synthesis had ceased in these mature animals. The weight per nucleus in these same animals increased gradually with age. Such a slight but continuous increase might be an indication that the fibers of the left ventricle were increasing in size during growth.

It would seem that after puberty, the growth of the left ventricle of the aerobic group 25 week of age enlarged solely by an increase in cell size. This was seen by an increase (P 0.05) in the weight per nucleus in these animals when compared to the A14 and A33. These findings agreed with the results of other researchers (Enesco and Leblond, 1962; Winick and Noble, 1965; Grimm et al., 1966; Korecky and French, 1967; Matsumoto and Krasnow, 1968; Sasaki et al., 1968 a, b). Although the differences with age were not significant, the observed progressive decrease in the DNA concentration up to 25 weeks would imply that the cells of the left ventricle were increasing in cell size with age.

The left ventricular DNA content and total number of nuclei were not influenced by an aerobic and an anaerobic training programs as shown in this study (Figures 4.1 and 4.3 respectively). This was in accordance with other studies (Bell et al., 1975; Sordahl et al., 1977).

In contrast, the A25 demonstrated a significant decrease in the DNA concentration and a significant increase in the weight per nucleus following 20 weeks of aerobic training (Figures 4.2 and 4.4 respectively). These two variables, as mentioned previously, were indication of changes in cell size. According to Matsumoto and Krasnow (1968), the DNA concentration was inversely related to cell size, if a constant diploid DNA content per cell was assumed. Enesco and Leblond (1962) stated that the DNA content in a single diploid nucleus of the rat's heart was constant and contained approximately 6.2 pgm of DNA. They have also pointed out that the weight per nucleus was an index of cell size. Any increase in cell size as a result of an enlargement of the nucleus and/or cytoplasm would increase the weight per nucleus.

Therefore, it can be concluded that the cell size of the left ventricle of the A25 was increased above the control level. Since the adjusted left ventricular weight and the protein content of the A25 (Figures 1.5 and 2.1 respectively) were not significantly different from the control values (P 0.05), the findings of the present study cannot confirm definitely if hypertrophy of the left

ventricle had occurred. The trend toward a larger left ventricle of the A25 was nevertheless evident.

5- LEFT VENTRICULAR HYDROXYPROLINE CONTENT AND CONCENTRATION

The connective tissue of the cardiac muscle can be referred to as the non-muscular proteins which is made up totally of collagen. This protein contained the amino acid proline, which is rarely found in proteins other than collagen. The role of collagen in determining the aging process of an organ has been considered since it is believed than an increase of crosslinkings of the collagen macromolecules also occurred with aging (Chvapil et al., 1964; Sasaki et al., 1976). Therefore, chemical determination of hydroxyproline make it possible to determine the collagen and thus the connective tissue changes of the left ventricle.

The role of connective tissue represented by collagen, in aging is still unknown. The results from various studies are controversial. While von Knorring (1970) has reported no increase in the collagen content with aging, Tomanek et al. (1972) have demonstrated a definite increase inhhydroxyproline concentration in rat's heart with age. Kiiskinen and Heikkinen (1976) have also found that the hydroxyproline concentration in the heart tissue of mice increased with age (39% over 19 weeks).

Findings from the present study revealed that the hydroxyproline content and concentration increased significantly with age both in control and trained animals (79%

over 28 weeks). The control animals between 14 weeks to 33 weeks of age showed a continuous but lower rate of increase in hydroxyproline content while the trained animals of the same age showed a considerable increase (P 0.05). This rate of increase was somewhat more pronounced (P 0.05) in the anaerobic animals (Figures 5.1 and 5.2 respectively).

A significant growth changes in the hydroxyproline content in the left ventricle of the anaerobically trained animals was noticed with advanced aging. While the results of the present study indicated that the hydroxyproline content in the left ventricle did not differ significantly in the trained animals as compared to their age-matched controls and to the aerobically trained animals, mean value for this variable was higher in the 33 week-old anaerobically trained animals. This progressive rise in the hydroxyproline content in these animals would tend to indicate that aging associated with the intensity of the training program influenced to a larger extent the left ventricular elasticity and compliance when compared to the aerobically trained animals.

The observed increase in the heart weight following 20 weeks of aerobic training was not associated with an increase in the hydroxyproline content and concentration. This increase in the cardiac mass of these animals would appear to be correlated with an increase in hydroxyproline content and concentration as a result of aging rather than training. This finding substantiate the results of Tomanek

et al. (1972) in hearts of male rats and Kiiskinen and Heikkinen (1976) in hearts of male mice. Therefore, changes in the collagen content and concentration in the left ventricle of male rats 25 and 33 weeks of age seemed to be affected by age and that neither training program altered this age-associated enhancement.

The aerobic training decreases significantly the hydroxyproline content of the left ventricle of the A14 when compared to the age-matched control group C14. This decrement in hydroxyproline content was not expected as a result of moderate aerobic training. According to Chvapil et al. (1973) and Bartosova et al. (1969) when young rats were subjected to an intensive training programs, the content of collagen in the heart muscle increased significantly over the control levels. They considered the young age of the animals to be somewhat related to this increase, since no changes in the collagen content were observed in older animals submitted to the same training programs. Other studies have reported no change in the hydroxyproline content and concentration in young rats undergoing mild to moderate training programs (Tomanek et al., 1972; Steil et al., 1975; Dowell et al., 1976 a; Kiiskinen and Heikkinen, 1976). The findings, from this study, that moderate aerobic training in pre-pubertal animals decreased the connective tissue considerably may imply that the ventricular elasticity and compliance of these animals were affected, and that the breakdown of collagen occurred.

There seem to be no rational explanation at the present time to explain the observed decrease in hydroxyproline content in pre-pubertal animals after a moderate aerobic training program and the need for more research in this area seems to be justified.

Summary and Recommendations

The present study investigated the effects of anaerobic and aerobic training programs extended over several months on selective cardiac parameters in the left ventricle of male rats.

1- A significant growth effect was observed in animals 5 to 14 weeks of age in almost all variables investigated, except the RNA to DNA ratio and the RNA concentration. The magnitude of change was more pronounced in prepubertal animals (5 to 14 weeks of age) as compared to postpubertal animals (25 to 33 weeks of age). There seems to be a need for further research studying the differential effects of similar training programs in postnatal animals up to puberty.

2- Nine (9) and twenty (20) weeks of aerobic training significantly reduced the body weights of the trained animals when compared to their age-matched control. There seemed to exist a general concensus among researchers of the influence of aerobic training in reducing the rate of growth of body weights in male rats.

3- Twenty (20) weeks of aerobic training seemed to have induced cardiac hypertrophy. Even though the post hoc test did not reveal significant mean differences in the adjusted left ventricular weight in the aerobic trained group, there existed evidence that the left ventricular fibers had increased in size. The training program elicited

a significant decrease in the DNA concentration and a significant increase in the weight per nucleus implying that increase in the size of the left ventricular fibers had occurred. Since the adjusted left ventricular weight was not significantly different from the control weight the findings of the present study cannot definitely confirm that cardiac hypertrophy of the 25-week aerobic trained group had occurred. However, the trend towards a larger left ventricle was demonstrated.

4- The selected training programs of varying intensities and duration, adopted in the present study influence somewhat the growth pattern of the left ventricular nucleic acids by maintaining previous existing levels with advanced aging. If drastic changes are to be expected in these biochemical parameters as related to training, the stimulus must be applied very early in life when protein synthetic activity is highly elevated. During the early growth period the DNA and RNA might be more susceptible to change under more severe physical stress.

5- There is a need for further research in establishing the effects of training programs of different intensities and duration on the connective tissue in the heart of young animals. The observed decrease in the hydroxyproline content after 9 weeks of aerobic training was not expected and cannot be explained at the present time.

The observed results seem to indicate a consistent tendency towards a greater improvement in some of the variables

investigated following 20 weeks of aerobic training. Further training does not seem to overload or stress sufficiently the cardiac muscle to adapt above the already attained functional level. Similarly, it seemed that high intensity sprint training does not elicit changes in any of the variables measured. This would imply that the duration and the amount of exercise rather than the intensity of training elicited selected ventricular adaptations in response to chronic endurance exercise.

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APPENDIX A-I

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

I- NORMAL GROWTH AND DEVELOPMENT OF THE HEART IN RATS

Hypertrophy and hyperplasia are two alternatives modes of growth of an organ. That is, the growth can proceed by an enlargement of already existing cells or by an increase in the number of cells or by a combination of both (Goss, 1964; Goss, 1966; Poupa et al., 1970; Brown, 1971).

These two phenomena are organ-specific. The organs can be placed under functional demands that can alter the growth pattern differently. This will depend on the quality of the stimulus that induces growth and the time of ontogeny upon which such stimulus is applied.

The hypertrophic and/or hyperplastic growth of the heart exhibit an interesting sequence of changes during normal growth from the embronic stage, to birth and to the stage of maturity in the rats (Goss, 1964).

A- PRENATAL GROWTH AND DEVELOPMENT

Autoradiographic studies of the hearts of 14 and 17 day-old fetal rats have shown a 35% and 60% increase in labelling of the cardiac muscle nuclei respectively, after 4 hours of administration of Thymidine-(H^3) (Rumgantsev, 1964). It seemed that after conception, growth proceeded entirely by cell division represented by an increase in the weight, total protein and total DNA content of the heart (Winick and Noble, 1965).

These studies suggested that the prenatal growth of

the heart was undergoing mitosis or cell division of its nuclei, i.e. cell hyperplasia and to a lesser degree cell hypertrophy (Enesco and Leblond, 1962).

B- POSTNATAL GROWTH AND DEVELOPMENT

1- HYPERTROPHY, HYPERPLASIA AND PROTEIN SYNTHESIS

There seemed to exist little doubt that the cardiac muscle cells continue to divide mitotically for some time after birth especially in the neonatal period. The activity appeared to be between 20 to 65 days (Goss, 1964; Winick and Noble, 1965; Brown, 1971; Zak, 1973).

This is coincident with the DNA content which increases more rapidly than the weight of the heart, confirming the increment in cell numbers (Enesco and Leblond, 1962; Sasaki et al., 1968 a).

Results from a recent study indicated that (H^3) Thymidine incorporation into DNA in cardiac muscle of the rat ceased completely by the 17th day of postnatal development (Claycomb, 1975). It was concluded that in the terminally differentiated ventricular cardiac muscle cell, the DNA synthesis and mitosis were irreversibly repressed and can occur no longer during the remaining life span of the animal. This has also been suggested previously by other workers (Morkin and Ashford, 1968; Grove and al., 1969 b; Skosey et al., 1972).

It was thought that the decrease in cytoplasmic DNA polymerase was the mechanisms regulating activity in the myocardium (Doyle et al., 1974; Claycomb, 1975). This enzyme

seemed to parallel temporarily the reduced rate of DNA synthesis between day 1 to day 17 after birth. Thereafter, the myocardial cell enlargement overpowered cell proliferation and became the principal process by which the heart as a whole enlarged (Zak, 1973; Jaweed et al., 1974).

Some investigators still favor an increase in muscle cells in growth and in hypertrophy during the postnatal period (Grimm et al., 1966; Korecky and French, 1967; Sasaki et al., 1968 a,b). These researchers have found that the cardiac DNA content increases as the heart enlarges. Assuming that the DNA content per nucleus was constant (Sasaki et al., 1968 b) the only conclusion that can be reached was that the number of cells had also increased.

On the other hand, Matsumoto and Krasnow (1968) have found that the DNA concentration in the left ventricles decreased with increasing heart weight, indicating an increase in cell size of this ventricle during normal growth of the rat.

The RNA per nucleus of cardiac cell varied with individual organ and seemed not to be affected by age (Winick and Noble, 1965). There exist a high RNA to DNA ratio even during rapid cell division of the heart. It would appear that RNA obtained its final content per nucleus or per cell in early growth of the organ and was sufficient to sustain normal rates of protein synthesis in normal growth. Thereafter the amount of RNA remained unchanged (Winick and Noble, 1965).

2- MYOCARDIAL CELLS

The cardiac muscle cell underwent a 3-fold increase in cell size during postnatal development, from about 5 to 6u at birth (Zak, 1973) to 14 to 18u in the adult heart (Rakusan and Poupa, 1963).

The diameter of the muscle cell of the ventricles of different mammals seemed to be the same irrespective of the size of the heart. But the number of myocardial cells varied directly with the size of the heart (Zak, 1973), i.e. the greater the heart size, the greater the absolute number of cardiac cells.

The weight of the left ventricle was normally around twice the weight of the right ventricle coincident with the adaptive response to the increase in workload after birth (Goss, 1971). The decrease in the right ventricle weight relative to the total cardiac mass was probably due to the decreased in pulmonary pressure seen in the neonatal period (Carney, 1969); Matsumoto and Krasnow, 1968). The difference in size of the muscle fiber after birth between the two ventricles seemed to be nothing more than a growth response to a regularly occurring physiological adaptations.

3- MYOCARDIAL CAPILLARIES

An adequate supply of blood is obviously necessary for cardiac growth, normal and hypertrophic as well as for optimal myocardial function in delivering the oxygen and the required nutrients to satisfy the demand of the working muscles.

Regarding the evidence supporting the hypothesis

that there was no increase in the number of cardiac muscle cells during postnatal growth, there seemed to exist an increase in the number of capillaries during the same period of ontogeny (Goss, 1964; Rakusan et al., 1965). This was attributed to a proliferation of capillaries during a time when the muscle fibers were increasing in size (Rakusan et al., 1965). The diffusion distance increases in relation to heart growth and seemed to be shortest in rats 20 to 23 day-old (Rakusan and Poupa, 1963).

Whereas, in the adult rat, the number of muscle fibers and capillary density gradually decreased as a result of the growth of the diameter of muscle fibers and the capillary to fiber ratio remained constant i.e. one to one (Rakusan and Poupa, 1963; Rakusan et al., 1965). This observation had also being reported in humans (Linzbach, 1960).

In the heart muscle of old animal (22 months and over), the capillary density was lower whereas the number of muscle fiber per surface area was unchanged. The result of such changes was a significant decrease in the capillary ratio and a prolongation of the diffusion distance (Poupa et al., 1970).

It was earlier assumed that beyond a critical size the growth of the cardiac muscle cells exceeded that of their accompanying blood vessels (Goss, 1964). This alteration could lead to serious problems of metabolic exchange due to an increase in the diffusion distance.

Evidence in cardiomegalies due to chronic exercise,

has indicated that vascularization did keep pace with hypertrophy of the heart muscle (Tepperman and Pearlman, 1961; Stevenson et al., 1964).

4- MYOCARDIAL CONNECTIVE TISSUE

During the period of growth and development after birth the enlargement of the cardiac muscle seemed to be confined to the proliferation of connective tissue, endothelial and other non muscle cells (Sasaki et al., 1968 a; Brown, 1971; Zak, 1973).

The role of collagen represented by connective tissue, in aging is still unknown, and the results from the reported observations in rats are also controversial.

While von Knorring (1970) have reported no increase with aging in the collagen content in the rat heart, Tomanek et al. (1972) have demonstrated a definite increase in hydroxyproline content in the rat heart with age.

Hence, the correlation between aging and collagen in the heart muscle of the rats is still not settled (Sasaki et al., 1976).

In experimental produced cardiac enlargement the DNA content in the mature heart did increase (Nair et al., 1971). This increase seemed to be the result of proliferation of non muscle cells (Grove et al., 1969 b) since only 3 to 5% of polyploidization occurred (Grove et al., 1969 a) and this small amount was considered to be negligible to the increment observed in the DNA content (Nair et al., 1971).

Therefore, the bulk of the increase in DNA content

in experimental induced cardiac enlargement is from cells other than the cardiac muscle cells in nature rats.

II- ADAPTIVE GROWTH OF THE HEART IN RESPONSE TO EXERCISE

A- GENERAL CONSIDERATIONS

One of the main determinants of cardiac size, both in the developing and adult animal is the work load placed upon it. This stimulus greatly depends on the stage of development of the heart at the time it is given (Zak, 1973). For example a work load imposed on the early neonatal heart resulted in an enlargement characterized by an increase in both the number and size of the myocardial cells (Bloor et al., 1968; Zak, 1973). Whereas the adult heart only enlarges as a result of an increase in the number of its components (Jaweed et al., 1974) i.e. the connective tissue (Morkin and Ashford, 1968; Grove et al., 1969 a,b).

Cardiomegaly has been shown to occur experimentally as the result of thyroxine administration (Edgrem et al., 1976) nutritional anemia (Korecky and French, 1967) aeortic constriction (Nair et al., 1968; Dowell et al., 1976 a,b) and many others (Bartosova et al., 1969).

These cardiac induced enlargement studies are considered to be appropriate models for pathological heart growth. The increase in myocardial mass in such studies was the result of multiplication of myocardial fibers as well as an increase in size (Crews and Aldinger, 1967). In such pathological conditions the cavity was usually dilated and was referred to as eccentric hypertrophy (Jaweed et al.,

1974, Linzbach, 1960).

On the other hand, highly active persons, such as athletes and laborers tended to have larger hearts than non-athletes and/or sedentary individual and it seemed to be a results of increase demand due to work (Linzbach, 1960; Poupa et al., 1970; Jaweed et al., 1974). The heart was usually found to be enlarged due to changes in its wall thickness, i.e. an increase in the size of the myocardial fiber (Young, 1970) with a concomitant alterations in its functional properties (Crews and Aldinger, 1967; Penparkgul and Scheuer, 1970; Ford, 1976).

This type of cardiac enlargement is a characteristic of all physiologic enlargement (Jaweed et al., 1974) also known as work hypertrophy (Crews and Aldinger, 1967) and/or exercise hypertrophy (Ford, 1976).

Whether or not the enlarged heart is detrimental because of an increase in the diffusion distance for oxygen and metabolites is still unclear and has not been thoroughly elucidated. Some studies do support the hypothesis that the hypertrophied heart is more powerful than the normal heart (Crews and Aldinger, 1967; Penparkgul and Scheuer, 1970; Steil et al., 1975) when cardiac enlargement is induced by exercise.

However, in experimental produced cardiac enlargement, evidence to support the hypothesis that the hypertrophied heart might be functionally inferior was self-explanatory by such models simulating pathological cardiac enlarge-

ment (Dowell et al., 1976 a).

The nucleic acids, protein and connective tissue of the myocardium in response to simulated pathological conditions have been well documented elsewhere (Badeer, 1972; Rabinowitz and Zak, 1972).

B- CARDIAC ENLARGEMENT DURING EXERCISE

Cardiac induced enlargement as a result of chronic strenuous exercise has been a subject of great interest since early in this century (Haiti, 1915; Vanliere and Northup, 1957; Tepperman and Pearlman, 1961; Stevenson et al., 1964; Vanliere et al., 1965; Crews and Aldinger, 1967; Arcos et al., 1968; Bloor et al., 1968; Aldinger, 1970; Bloor and Leon, 1970; Leon and Bloor, 1970; Penpargkul and Scheuer, 1970; Poupa et al., 1970; Oscai et al., 1971 a; Oscai et al., 1971 b; Tomanek et al., 1972; Jaweed et al., 1974; Steil et al., 1975).

The stimulus that produced the dramatic alterations in the structure and function of the myocardium is still unknown (Badeer, 1972; Meerson et al., 1974). It seemed that the mechanism regulating the cardiac enlargement induced by exercise is in direct contrast with the cardiac enlargement mechanisms employed during experimentally induced cardiomegalies (Dowell et al., 1976 a).

Cardiac enlargement is considered to be an important adaptive mechanism whereby the heart is able to sustain the increase circulatory load produced by exercise (Young, 1970).

1- AGE AND SEX OF THE ANIMALS

The effects of age on the various functions of the myocardium have been extensively reviewed elsewhere (Gerstenblith et al., 1976).

It is important to consider the age of the animals when determining the cause for the cardiac enlargement, because of the peculiar sequence of growth and development unique to the heart at different periods of development (Goss, 1964).

Bloor et al., (1968) have shown that mild to moderate swimming produce cardiac enlargement in 4 week-old animals after 10 weeks of swimming one hour twice a week and one hour every day respectively. It was suggested that the enlargement was a result of an increase in the number of myocardial cells (hyperplasia). In another study, using the same swimming programs, Bloor et al., (1970) have concluded that the hearts of young rats increase significantly in weight under mild physical stress which didn't produce cardiac enlargement in older rats submitted to the same program.

When young rats (2 months old) were subjected to run 24 meters per minute for 30 minutes spaced by 30 minutes rest repeated 6 times every day for 7 weeks, the content of collagen in the heart tissue increases due to the intensity of the training program (Bartosova et al., 1969; Chvapil et al., 1973). When older rats (8 months old) were submitted to the same training program, no change in the content of collagen was reported. The investigators suggested that physical activity affected the growth pattern of collagen

significantly in young rats.

A recent study, using 3 week-old rats and submitted them to swim one half hour every day has shown no change in heart weight after 3, 6 and 9 weeks of swimming (Bell et al., 1975). They have also reported that the swimming program didn't alter the growth rate and development of the heart. This was also substantiated by Houston and Green (1975) where they have demonstrated that the experimental animals that ran 24 minutes at 50 meters per minute on a 12% grade, their hearts were not significantly different in weight than the control hearts in rats 3 to 4-week-old. A consistent tendency was observed towards lower heart weight, although, not significant.

It would appear that the physiological stimulus of physical exercise is most effective very early in life. The ideal period seemed to be between the ages of one and five weeks after birth (Bell et al., 1975).

In adult rats' studies, a more intensive training program is favorable in producing any change in the heart weight (Bloor and Leon, 1970).

The training programs given to male rats, regardless of age, tended to decrease significantly the body weight when compared to control animals. This reduced body weight increased the heart weight to body weight ratio (heart ratio).

Most studies on adult animals were done on female rats (Vanliere and Northup, 1957; Crews and Aldinger, 1967; Oscai et al., 1971 a; Jaweed et al., 1974). It seemed that

the body weight was less affected by the intensity of the training program (Oscai et al., 1971 b) as demonstrated in the heart weight which increased significantly over the control hearts.

In older rats (46 to 50 weeks old) subjected to a moderate swimming program for 10 weeks had their body weights and ventricular weights significantly decrease as compared to the control group of the same age (Bloor et al., 1968). The heart ratio of such rats was not altered even though there seemed to be a loss of myocardial mass.

When similar training programs were given to both male and female rats, the latter exibited the greater degree of cardiac enlargement (Vanliere and Northup, 1957). Also when female and male rats were subjected to an identical swimming program the female rats had a greater increase in heart weight to body weight ratio (Oscai et al., 1971 b). It was suggested that the difference in the observed cardiac enlargement was on account of the reduced body weight demonstrated by the male rats (Oscai et al., 1971 b).

Jaweed et al. (1974) have used an adapted training program from Pattengale and Holloszy (1967) and have reported an increase in the heart ratio in female rats. Dowell et al. (1976 b) have also employed a modification of the Pattengale and Holloszy's training program. Using female rats, cardiac enlargement was not produced by such a running program of moderate intensity. The authors have suggested that female rats were not willing to perform treadmill exercise of the

intensity and duration used with male rats (Dowell et al., 1976 b).

It would appear from these studies that the effects of exercise on the myocardium greatly depend on the age of the animal. If changes of the myocardium components in adult rats are to occur, the intensity and the duration of the exercises have to be augmented accordingly.

2- TYPES OF TRAINING PROGRAMS

Training programs used with small animals in the study of the heart response to exercise have ranged from voluntary running on a revolving wheel (Haiti, 1915; Jaweed et al., 1974) to forced-running on a motor driven treadmill (Vanliere and Northup, 1957; Vanliere et al., 1965; Oscai et al., 1971 a; Houston and Green, 1975; Jaweed et al., 1974; Dowell et al., 1976 a; Dowell et al., 1976 b); from swimming in a tank with additional weights attached to their tails (Crews and Aldinger, 1967; Bloor et al., 1968; Bloor and Leon, 1970; Oscai et al., 1971a; Oscai et al., 1971 b; Jaweed et al., 1974; Bell et al., 1975) to weightlifting by climbing a ladder carrying weights on their backs (Jaweed et al., 1974).

a- INTENSITY

Mild swimming exercise (one hour twice weekly for 10 weeks) did not produce cardiac enlargement in young rats (Bloor et al., 1968) but when the animals were subjected to one hour daily for 10 weeks, cardiac enlargement was induced by the chronic exercise. In another study where the male

animals were swimming a half hour every day for 9 weeks, no change in the heart weight was reported (Bell et al., 1975). In a recent study, young male rats were submitted to a running program on the treadmill at a speed of 10 meters per minute for 30 minutes per day for 4 weeks. No differences were reported in body weights and in heart weights. There was a consistent tendency (although not significant) towards lower values in the exercised animals when compared to the control animals (Dowell et al., 1976 a).

b- DURATION

In terms of the duration of the training programs, studies were conducted as short as 3 weeks (Crews and Aldinger, 1967; Bell et al., 1975) anf for as long as 10 weeks (Bloor and Leon, 1970).

In the former studies the training program consisted of swimming six hours per day, six days per week and one half hour every day respectively. Crews and Aldinger (1967) have found a significant increase in heart weight whereas Bell et al. (1975) have reported no cardiaccenlargement. Bloor and Leon, (1970) have subjected their animals to swim one hour twice weekly for 10 weeks and the swimming program didn't have any effects on the heart weight and consequently no cardiac enlargement was observed.

Obviously, the intensity of the training program seem to be the common factor in producing cardiac enlargement in response to exercise.

C- SELECTIVE CARDIAC PARAMETERS MEASURED IN RESPONSE TO EXERCISE

1- MYOCARDIAL WEIGHT

Since the first study on the effects of exercise on the cardiac muscle weight (Haiti, 1915), numerous studies have been undertaken (Vanliere and Northup, 1957; Vanliere et al., 1965; Crews and Aldinger, 1967; Oscai et al., 1971 b; Jaweed et al., 1974; Dowell et al., 1976 b). Such studies have used the heart weight as an indicator of cardiac enlargement. Consequently, a heart ratio (heart weight to body weight ratio) was frequently used to establish if an alteration in the heart growth had occurred as a response to chronic exercise. This practise was criticized by Heroux and Gridgement (1958). A regression technique was suggested to normalize the heart weights for differences in body weights at the time of sacrifice since it was shown that the heart growth didn't not parallel the body growth when subjected to exercise (Oscai et al., 1971 b) as normally observed in control young animals (Dunn, 1947; Ostadal et al., 1967).

In exercised male animals, the rate of growth as indicated by the body weights seemed to be influenced by exercise. In most cases the exercised male animals demonstrated a less weight gained when compared to free eating control male animals (Haiti, 1915; Vanliere and Northup, 1957; Oscai et al., 1971 b; Bloor et al., 1968; Bloor and Leon, 1970; Houston and Green, 1975; Dowell et al., 1976 a). According to Oscai et al. (1971 a) male rats that exercised

vigourously tended to demonstrated an appetite-suppressing effect as noticed by a reduction in food intake (Crews et al., 1969; Oscai et al., 1969).

This condition appeared not to be the case in exercise female rats where no significant changes in body weight were found following chronic exercise when compared to the control female animals (Crews and Aldinger, 1967; Arcos et al., 1968; Oscai et al., 1971 a; Jaweed et al., 1974) and when compared to the exercise male animals (Vanliere and Northup, 1957; Oscai et al., 1971 b).

Cardiac enlargement was suggested to have taken place due to the significant increase in the heart's ratio (Jaweed et al., 1974).

In relation to this ratio, there appeared to be a positive correlation between heart weight and maximum cardiac output (Arcos et al., 1968; Beznak et al., 1958). Hence, an increase in the heart weight to body weight ratio in exercise animals can thus, be associated with an increase in the maximum capacity to deliver blood to the exercising muscles. This would be more beneficial if the skeletal muscles would not significantly increase in weight relative to its body weight. Hence, studies on skeletal muscle involving excercises (swimming and running) and also strenght exercises (i.e. weightlifting) didn't produce skeletal muscle enlargement (Holloszy- 1967; Oscai et al., 1969; Jaweed et al., 1974; Hickson et al., 1976).

Such studies revealed that when assessing a true cardiac enlargement in male animals the reduced body weight must be taken into consideration.

2- MYOCARDIAL NUCLEIC ACIDS AND PROTEIN CONTENT

Investigators have substantiated the fact that the cardiac muscle enlarges in response to chronic exercise as shown with a concomitant increase in the heart weight to body weight ratio.

The increase in DNA content of the developing heart was found to be almost proportional to the increase in heart weight (Morkin and Ashford, 1968). The increment observed in the heart weight in response to exercise would necessitate a concomitant increase in the nucleic acids of the myocardium as expressed in the DNA and RNA content and concentration.

However, few studies (Bell et al., 1975; Dowell et al., 1976 a; Dowell et al., 1976 b) have attempted to evaluate the effects of chronic exercise on the protein synthesis of the cardiac muscle.

Such studies were conducted in young male (Bell et al., 1975; Dowell et al., 1976 a) and female (Dowell et al., 1976 b) rats.

One study (Bell et al., 1975) looked on the effects of chronic exercise on the growth pattern of the cardiac muscle. In this investigation, 3-week-old male rats were subjected to a swimming program of one half hour per day, every day for 3, 6 and 9 weeks of duration. The training

program didn't alter the growth pattern of the developing rats and interestingly enough didn't produce cardiac enlargement. The heart weight, myocardial DNA and RNA content and the total myocardial protein content increased significantly during the first nine weeks of growth, both in control animals and in exercised animals. After this period the paremeters measured, stayed fairly constant and no modification was found between the control and the exercised animals at any given time during the swimming program.

The investigators (Bell et al., 1975) have suggested that in order for any exercise program to influence myocardial nucleic acid concentration or protein content, the exercise must be applied for as long a period as possible before the onset of puberty. Suggesting the importance of the age of the animals.

Also in studies using young female (Dowell et al., 1976 b) and male (Dowell et al., 1976 a) rats, no significant changes were observed in the myocardial levels of DNA and RNA after chronic of similar intensity i.e. 25 meters per minute for 60 minutes per day, 5 days per week for 8 and 11 weeks of duration respectively.

Since no significant changes were found in the nucleic acids level in the exercise animals (Bell et al., 1975; Dowell et al., 1976 a; Dowell et al., 1976 b), it was unlikely that the adaptive response represent an increase in myocardial protein synthetic activity above normal levels.

3- MYOCARDIAL CONNECTIVE TISSUE

The supporting elements of the cardiac muscle can be referred to as the non-muscular proteins, eg. the heart's connective tissue, and, also is believed to be the only protein in mammalian tissue that contains the imino acid, hydroxyproline (hydro).

The role of collagen in determining the aging process of an organ has been considered since it was believed that an increase of crosslinkings of the collagen macromolecules also occur with aging (Chvapil et al., 1964; Sasaki et al., 1976). But results reported were inconsistent as mentioned earlier in the review.

In experimentally induced cardiac enlargement, the increase in myocardial DNA synthesis was suggested to represent the increase in the normal synthesis occurring in interstitial cells and not in the synthetic activity within the myocardial cells (Morkin and Ashford, 1968).

No increase in the content of the myocardial collagen was found when thyroxine was administered to produce cardiac enlargement (Edgren et al., 1976) such was not the case in other forms of experimental cardiomegalies (Buccino et al., 1969; Grove et al., 1969 b; Bartosova et al., 1969; Skosey et al., 1972). It seemed that the bulk of the DNA synthesis during such experiments was associated with mitotic activity in the less differentiated elements of the cardiac tissue (Grove et al., 1969 a). Goss (1966) has suggested that the connective tissue elements possessed a greater

potentiallity for hyperplasia than striated muscle. This was also substantiated by Morkin and Ashford (1968), Grove et al. (1969 a) and Skosey et al. (1972), that the connective tissue cells of the adult heart of the rat in similar investigations, proliferated rapidly and occurred almost exclusively in connective tissue cells.

When young rats (2 months old) were subjected to chronic exercise, the content of collagen in the heart tissue increases due to the intensity of the training program (Bartosova et al., 1969; Chvapil et al., 1973). From these two studies, the experimental animals were running at 24 meters per minute for 3 hours, running for 30 minutes spaced by 30 minutes of rest for 6 weeks and 9 weeks respectively. When older rats (8 months old) were submitted to the same training program, no change in the content of collagen was reported. The investigators have suggested that physical activity of such intensity has affected the growth pattern of collagen significantly in young rats.

In contrast to these reported observations, Tomanek et al., (1972), Steil et al. (1975) and Dowell et al. (1976 a) have shown no change in the content of collagen in rats heart in young (2 to 3 monghs old) and older rats (10 to 12 months old). The intensity of the training program was considered mild to moderate in nature, and lasted 4 to 12 weeks.

These reported studies suggested the importance of the intensity of the exercise when applied very early in life, if significant alterations in the connective tissue of

the heart were to occur.

III- CONCLUSIONS

It seems logical to conclude that the reported literature on the effects of chronic exercise on some selected parameters of the heart muscle, can be altered.

The myocardial nucleic acids seemed not to be influenced by any type of exercises.

The physiological stimulus (i.e. exercise) seemed to be more effective in early life than later when the animals approach senescence.

There seemed to be a need of an exercise program of very high intensity extended for several months on young rats on the selected cardiac parameters.

APPENDIX B

I- PURINA CHOW DIET

II- AEROBIC TRAINING PROGRAM

III- ANAEROBIC TRAINING PROGRAM

IV- TOTAL DISTANCE COVERED DURING TRAINING PROGRAMS

PURINA CHOW:

Crude protein not less than 23.0%

Crude fat not less than 4.5%

Crude fiber not more than 6.0%

Ash not more than 9.0%

Meat and bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, dried beet pulp, ground extruded corn, ground oat groats, soybean meal, dehydrated alfalfa meal, can molasses, animal fat preserved with BHA, vitamin B_{12} supplement, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, brewers' dried yeast, thiamin, niacin, vitamin A supplement, D activated plant sterol, vitamin E supplement, calcium carbonate, dicalcium phosphate, iodixed salt, iron sulfate, iron ocise, manganous oxide, cobalt carbonate, copper oxide, zinc oxide.

APPENDIX B-II

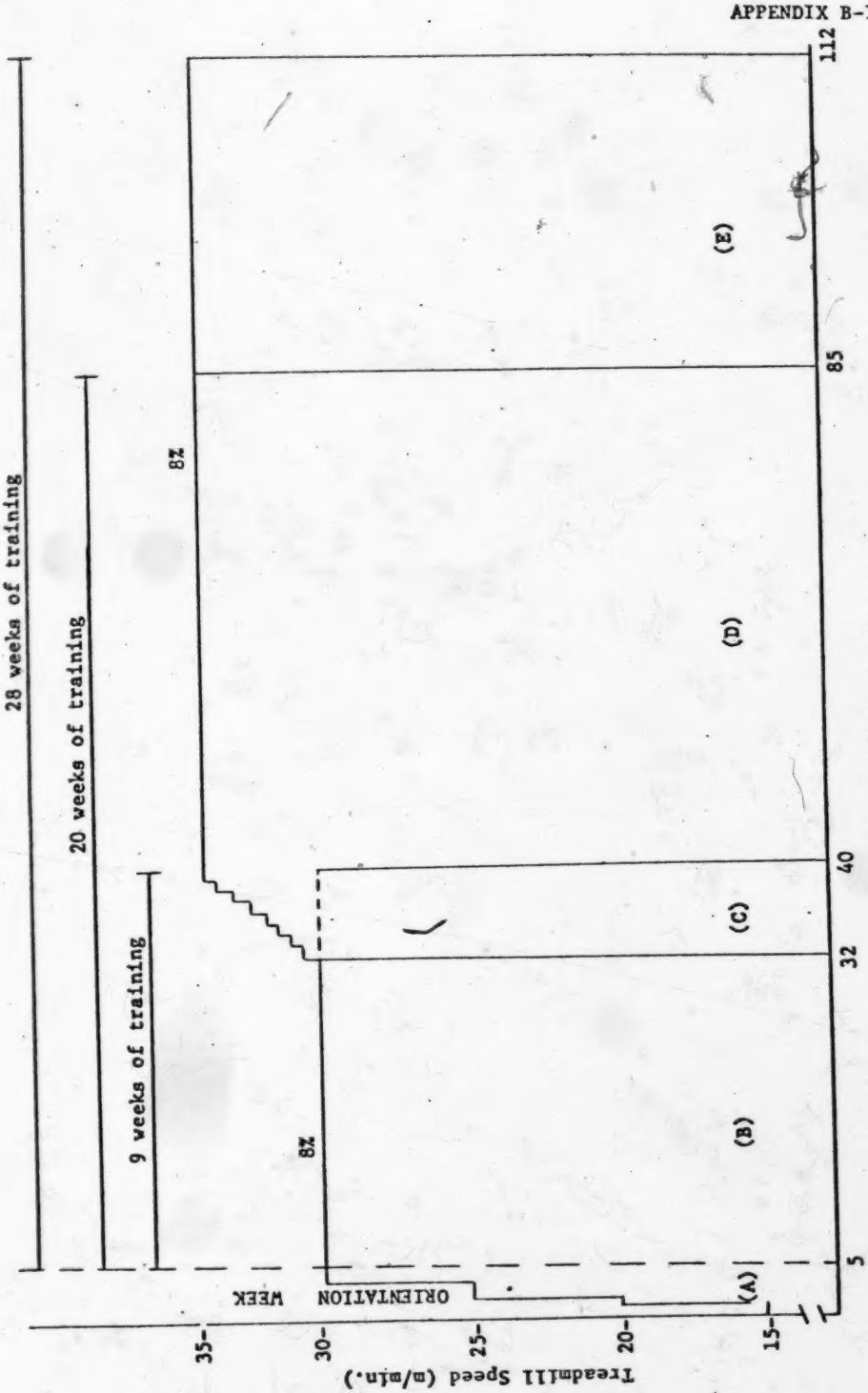

Figure 6.1- Training Protocol for the Endurance (aerobic) Training Group for 9, 20 and 28 Weeks.

APPENDIX B-III

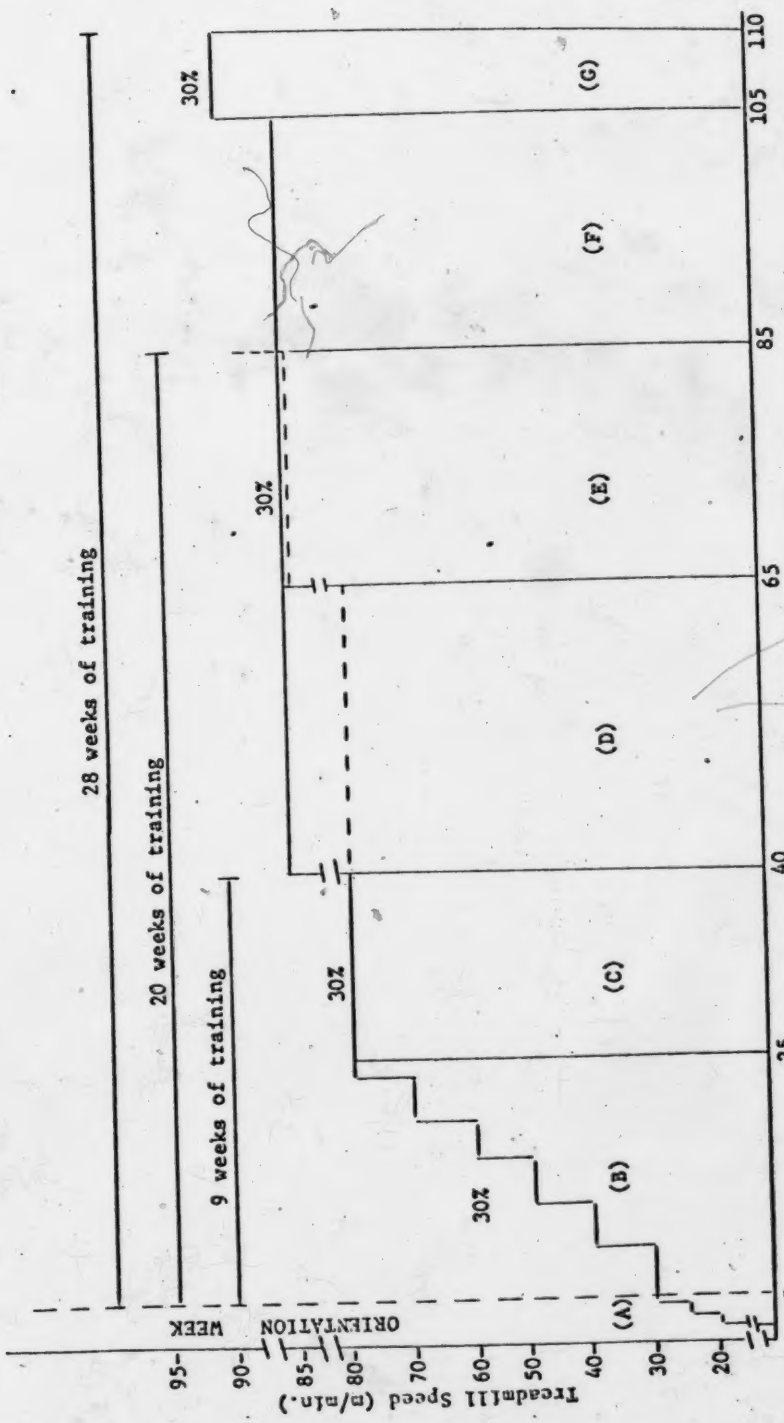

Figure 6.2- Training Protocol for the Sprint (anaerobic) Training Group for 9, 20 and 28 Weeks.

TABLE 3- Running Distance (in meters) for Aerobic Groups During the Entire Training Program *

	A	B	C	D	E	TOTAL+	%
9 weeks of training	1675	64500(+C)	-	-	-	66475	30%
20 weeks of training	1675	50400	15600	92400	-	160075	70%
28 weeks of training	1675	50400	15600	92400	67200	227275	100%

*for sections A to E, see Figure 6.1.

+final speed: 9weeks: 30 m/min.; 20 and 28 weeks: 35 m/min..

TABLE 4- Running Distance (in meters) for the Anaerobic Groups During the Entire Training Program*

	A	B	C	D	E	F	G	TOTAL+	%	%x
9 weeks of training	1675 (80)o	6700 (400)	6400 (320)	-	-	-	-	14775 (800)	30% 35%	22%
20 weeks of training	1675 (80)	6700 (400)	6400 (320)	9600 (480)	8500 (400)	-	-	32875 (1680)	70% 75%	21%
28 weeks of training	1675 (80)	6700 (400)	6400 (320)	10200 (480)	8500 (400)	8500 (400)	3600 (160)	45575 (2240)	100% 100%	20%

*for sections A to G, see Figure 6.2.

o( ) number of bouts.

x % of distance covered when compared to the aerobic group.

+final speed: 80m/min. for 9 week-group; 85m/min. for 20 week-group; 90m/min for 28 week-group.

APPENDIX C

BIOCHEMICAL PROCEDURES

I- PROTEIN DETERMINATION

II- NUCLEIC ACIDS EXTRACTIONS

III- RNA DETERMINATION

IV- DNA DETERMINATION

V- HYDROXYPROLINE DETERMINATION

PROTEIN DETERMINATION — APPENDIX C-I

The protein was measured by the biuret method as suggested by Layne (1957).

REAGENTS*	EQUIPMENTS
a- Cupric Sulfate ($CuSO_4.5H_2O$);	a- Test Tubes;
b- Sodium Potassium Tartrate ($NaKC_4H_4O_6.4H_2O$);	b- Pye Unicam Spectrophotometer
c- 10% Sodium Hydroxide (NaOH).	

1- PROTEIN STANDARD (BOVINE SERUM ALBUMINE - BSA)

Forty to sixty mg of the Bovine Serum Albumine was dissolved into 10 ml of re-distilled water making a final concentration of 4 to 6 mg per milliliter of standard protein solution.

2- PROTEIN DETERMINATION AND COLOR REACTION

0.5 ml of the homogenate was added with re-distilled water resulting to a final volume of 1.0 ml. To the protein solution, 4.0 ml of the biuret reagent was added. The purple coloured samples were left standing at room temperature for 30 minutes and were then read in a spectrophotometer at 550nm. The purple complex with Copper Salts in alkaline solution, making the biuret reagent, is form by substances (eg. animo acids) containing two or more peptide bonds.

3- PROTEIN STANDARD CURVE

Similar procedure, as above (2), was employed for the standard. The protein standards and blank (re-distilled

water) were analysed with each set of protein determination.

4-* BIURET REAGENT

The BIURET REAGENT consisted of dissolving 1.5 gm of Cupric Sulfate and 6 gm of Sodium Potassium Tartrate in 500 ml of re-distilled water. With constant swirling, 300 ml of 10% NaOH was then added. The reagent was diluted to 1.0 liter with re-distilled water and stored in a paraffin lined bottle. The reagent usually should keep indefinitely but must be discarded if contaminated.

5- SENSITIVITY

The sensitivity of the method is 0.25 to 200 mg.

NUCLEIC ACIDS EXTRACTIONS APPENDIX C-II

The DNA and RNA were extracted with a modified Munro and Fleck's (1966) modification of the Schmidt-Thannhauser's (1945) method.

REAGENTS

a- Perchloric Acid (PCA):

i- 10% PCA;

ii- 0.2N PCA;

iii- 0.6N PCA;

iv- 1.2N PCA;

b- Sodium Hydroxide (NaOH) (electrolyte pellets):

i- 0.3N NaOh.

c- Methanol (methyl Alcohol).

EQUIPMENTS

a- Sorval Superspeed RC2-B Automatic Refrigerated Centrifuge;

b- Vortex Jr. Mixer;

c- Polytron Homogenizer;

d- V.I.P. CO_2 incubator.

The frozen left ventricle was homogenized with a Polytron homogenizer in re-distilled water (1 part in 20 volumes). Duplicate of 2.0 ml aliquots of the homogenate were used for the extractions of the nucleic acids.

a- DNA and RNA extractions:

To these 2.0 ml aliquots:

i- Add 1.0 ml of 10% cold PCA, mix well in a Vortex;

ii- Add. 2.0 ml of methanol, mix well, and let stand on ice for 10 minutes;

iii- Centrifuge at 4000 x g for 20 minutes;

iv- Discard supernatant and mix pellet with a teflon rod;

v- Add 5.0 ml of 0.2N PCA, mix well in a Vortex and centrifuge at 4000 x g for 5 minutes (repeat twice);

vi- Add 4.0 ml of 0.3N NaOH to the pellet or precipitate and incubate for one hour at 37° in a V.I.P. cO_2 incubator;

vii- Add 2.0 ml of 1.2N PCA to the alkaline solution and centrifuge at 4000 x g for 20 minutes;

viii- Remove the supernatant (RNA FRACTION).

ix- Mix precipitate well with a teflon rod and add 5.0 ml of 0.2N PCA, mix well in a Vortex and centrifuge at 4000 x g for 5 minutes. Remove the supernatant (RNA FRACTION) and repeat twice;

x- To the well mixed precipitate add 4.0 ml of 0.6N PCA;

xi- Heat for 15 minutes in boiling water, let cool for 10 minutes after heating and centrifuge at 4000 x g for 15 minutes;

xii- Take 1.0 ml of the supernatant for the DNA determination (DNA FRACTION).

See Appendices C-III and C-IV for the RNA and DNA determination respectively.

RNA DETERMINATION APPENDIX C-III

The RNA contained in the extract and the washings were measured by the orcinol reaction for pentose as described by Schneider (1957).

REAGENTS

a- Calf Liver RNA

b- Ferric Chloride ($FeCl_3$):

c- i- 0.02% $FeCl_3$ dissolved in concentrated HCL;

c- Orcinol reagent*:

i- 6% orcinol.

EQUIPMENTS

a- Pye Unicam Spectrophotometer.

1- RNA STANDARD SOLUTION (CALF LIVER RNA)

Three to four mg of the Calf Liver RNA was dissolved in a 50 ml gradient flask with 0.3N NaOH. The standard solution was then incubated for one hour in a V.I.P. CO_2 incubator set at 37°C. Following the incubation, the standard was then placed in boiling water for 20 minutes. The final concentration of the standard solution was between 60 to 80 ugm per milliliter of standard RNA solution.

2- RNA DETERMINATION AND COLOR REACTION

A 1.0 ml of $FeCl_3$ (0.02%) and 0.1 ml of 6% orcinol were added to 1.0 ml aliquots of the RNA fraction (0.5 ml RNA: 0.5 ml re-distilled water) into screw cap tubes and placed in boiling water for 20 minutes. After cooling for 10 minutes the green coloured samples were read at 660nm in

a spectrophotometer. The orcinol reaction depends on the hydrolysis of the RNA (heating at 100°C) to yield furfural from the ribose and this then reacts immediately with orcinol to give an intensive stable green colour.

3- RNA STANDARD CURVE

To a volume of 2.0 ml with different concentrations of the RNA standard and re-distilled water, the RNA determination was analysed using the same analysis outlined above (2). RNA standards and blank (re-distilled water) were analysed with each set of RNA determination.

4- *ORCINOL REAGENT

The orcinol reagent was made daily prior to use and consisted of adding 1.0 ml of 0.02% Ferric Chloride and 0.1 ml of 6% orcinol to the RNA sample. The orcinol was dissolved with 95% ethanol.

DNA DETERMINATION APPENDIX C-IV

DNA contained in the extract was measured by the indole reaction for deoxypentose according to the procedure of Ceriotti (1975) as modified by Keck (1956).

REAGENTS

a- Hydrocholoric Acid (HCL):

i- 2.5N HCL;

b- Indole*

i- 0.06% indole.

EQUIPMENTS

a- International Clinical Centrifuge;

b- Pye Unicam Spectrophotometer.

1- DNA STANDARD SOLUTION (CALF THYMUS DNA)

Three to four milligrams of Calf Thymus DNA was dissolved in a 25 ml gradient flask with 3.0 ml of 0.3N NaOH and about 15 ml of 0.6N PCA was thereafter added. The standard solution was heated in boiling water for 15 minutes and cooled in ice for 10 minutes. The solution was then brought up to a final volume of 25 ml making a final concentration of 80 ugm of DNA per milliliter standard solution.

2- DNA DETERMINATION AND COLOR REACTION

A 0.5 ml volume of 2.5N HCL and 0.06% indole respectively, were added to 1.0 ml aliquots of the DNA fraction into screw cap tubes and placed in boiling water for 15 minutes. After cooling for 10 minutes in ice the hydrolyzate was then extracted twice with equal volume of iso-amyl acetate (in this case 2:2). After spinning for 10 to 20 secs in a clinical centrifuge, the suspended iso-amyl was then discarded and the yellow coloured samples were read

in a spectrophotometer at 490nm. The reaction of deoxyribose with indole in 2.5N HCL gives a yellow colour with a sharp absorption peak at 490nm.

3- DNA STANDARD CURVE

To a 1.0 ml volume of different concentration of the DNA standard with re-distilled water, the analysis was similar to the one described above (2). DNA standards and blank (re-distilled water) were analyzed with each set of DNA determination.

4- *INDOLE REAGENT

The indole reagent consisted of dissolving 0.06 gm into 100 ml with re-distilled water.

5- SENSITIVITY

The sensitivity of the method is 5ugm of DNA and even down to 0.2 or 0.1 ugm in micro modification.

HYDROXYPROLINE DETERMINATION — APPENDIX C-V

A modification of the method of Neuman and Logan (1950) was used to determine hydroxyproline.

REAGENTS

HYDROXYPROLINE EXTRACTION

a- Hydrochloric Acid (HCL):

i- 12N HCL;

b- Congo Red Indicator:

ii- 0.5 gm dissolved in 100 ml 25% ethanol;

c- Sodium Hydroxide (NaOH)

i- 3N NaOH;

d- NoritA (alkaline).

HYDROXYPROLINE DETERMINATION

a- Cooper Sulfate ($CuSO_4$):

i- 0.01M $CuSO_4$;

b- Sodium Hydroxide (NaOH):

i- 2.5N NaOH;

c- Hydrogen Peroxide (H_2O_2O):

i- 6% H_2O_2;

d- Sulphuric Acid (H_2SO_4):

i- 3N H_2SO_4;

EQUIPMENTS

a- Castle Orthomatic Autoclave;

a- Water Bath - Thelco;

b- Metabolic Shaking Incubator;

c- Pye Unicam Spectrophotometer.

e- Para-Dimethylamino Benzaldehyde (p-D.A.B.):

i- Twenty-five gm of recrystallized p-D.A.B. was made up with 500 ml of CPN propanol to make a 5% solution.

1- HYDROXYPROLINE STANDARD SOLUTION

The stock standard solution consisted of 0.1 gm Hydroxy-L-Proline in 200 ml re-distilled water making a final Hydroxyproline concentration of 500 umg per milliliter. This stock solution was stabled for up to 3 months under refrigeration.

2- HYDROXYPROLINE DETERMINATION AND COLOR REACTION

One ml of the samples of 1.0 ml each were done in triplicate. To the samples, the following reagents were added in sequence: 1.0 ml of 0.01M $CuSO_4$; 1.0 ml of 2.5N NaOH and 1.0 ml of 6% H_2O_2. The test tubes were mixed vigorously at room temperature for 5 minutes and then incubated into an 80°C water bath for another 5 minutes. The excess peroxide was destroyed by shaking vigorously during the incubation. The samples were cooled quickly in an ice-water bath. Four milliliters of 3N h_2SO_4 were added with agitation and then 2.0 ml of 5% p-D.A.B. were added. The samples were then incubated in a 70°C water bath for 16 minutes and then cooled in tap water. The pink coloured samples was read in a spectrophotometer at 540 nm.

3- HYDROXYPROLINE STANDARD CURVE

To a volume of 1.0 ml of different concentration of the hydroxy-L-proline stock solution (1, 2, 5, 10, 15,

20 ugm/ml as required) and re-distilled water, the analysis was conducted as above (2). Hydroxy-L-Proline and blank (re-distilled water) were analysed with each set of hydroxy-proline determination.

APPENDIX D

RAW DATA

FOR EACH DEPENDENT

VARIABLE

APPENDIX D-I

TABLE 5-Raw Data for Body Weights (BW), Heart Weights (HW) and Left Ventricular Weights (LVW) of the Different Groups of Animals

	BC	C14	AN14	A14	C25	AN25	A25	C33	AN33	A33
BODY WEIGHTS (grams)	124	430	436	314	500	525	481	546	411	332
	119	464	354	410	495	454	415	468	525	560
	123	435	362	348	468	534	453	413	495	477
	114	420	338	356	480	500	439	517	515	483
	117	378	392	396	568	454	444	559	463	355
	123	372	371	158	482	500	458	543	471	476
	119	402	407	388	465	471	469	515		
	122	428	289	334	518	506				
	117	411		351	532	456				
	123									
Means	120	416	369	339	500	489	451	509	480	447
±SEM	±1	±10	±16	±25	±10	±10	±8	±20	±17	±35
HEART WEIGHTS (mg)	482	1310	1165	1182	1203	1414	1379	1373	1083	1098
	489	1263	1080	1157	1321	1191	1205	1000	1282	1366
	525	1235	0956	1109	1278	1357	1229	1402	1284	1407
	417	1130	0887	0946	1190	1322	1165	1053	1329	1395
	454	0489	1210	1056	1329	1366	1308	1327	1192	1009
	464	1118	1035	0563	1518	1243	1241	1214	1116	1220
	490	1200	1121	1144	1200	1204	1389	1176		
	478	1276	0930	1100	1275	1385				
	442	1068		1102	1344	1193				
	490				1283					
Mean	473	1121	1048	1040	1294	1297	1274	1221	1214	1249
±SEM	±10	±85	±41	±64	±30	±30	±33	±59	±41	±69
LEFT VENTRICULAR WEIGHTS (mg)	259	735	708	593	714	784	761	671	580	612
	279	720	642	728	889	648	670	538	631	689
	284	707	528	631	742	745	654	653	678	721
	242	676	509	608	650	709	665	548	689	670
	245	565	699	576	672	638	688	653	653	513
	230	620	672	348	692	660	694	650	608	587
	219	623	693	669	649	661	769	603		
	265	780		602	675	680				
	240	640		608	695	633				
	246				718					
Mean	251	674	632	596	710	684	700	617	640	632
±SEM	± 7	±23	±28	±35	±22	±17	±18	±21	±17	±31

APPENDIX D-II

TABLE 6-Raw Data for Total Protein Content, Concentration and Protein to DNA Ratio of the Different Groups of Animals

	BC	C14	AN14	A14	C25	AN25	A25	C33	AN33	A33
TOTAL PROTEIN CONTENT (mg)	51	---	144	115	---	182	119	---	133	121
	59	163	154	---	168	163	171	130	145	182
	46	157	116	142	172	165	160	152	162	180
	--	142	122	133	152	167	140	160	175	---
	49	114	153	---	177	136	151	147	155	119
	--	127	134	---	154	154	162	152	135	142
	51	121	138	145	153	123	161	150		
	46	175	101	141	155	139				
	51	141		114	142	133				
					166					
Mean	51	143	133	132	160	151	152	149	151	149
±SEM	±1	±8	±7	±6	±4	±7	±7	±4	±7	±14
PROTEIN CONC. (mg/gm)	219	---	224	199	---	233	156	---	230	198
	226	241	259	---	189	251	255	242	228	264
	208	241	239	239	232	222	245	232	239	249
	237	218	261	230	233	235	210	292	255	---
	---	217	230	---	277	213	219	225	258	232
	228	218	238	---	226	233	234	234	243	272
	---	206	218	229	235	185	221	270		
	215	234	218	246	230	204				
	206	225		207	204	211				
	207				231					
Mean	214	225	236	227	239	233	230	264	251	263
±SEM	±4	±4	±6	±7	±8	±7	±14	±9	±3	±12
PROTEIN to DNA RATIO	86	---	---	187	---	202	190	---	137	140
	--	177	143	---	118	158	185	206	131	214
	96	128	144	178	154	168	220	177	175	201
	80	161	177	150	160	194	188	192	228	---
	--	103	192	120	199	190	213	174	222	156
	130	156	174	---	204	212	211	202	199	182
	87	172	202	196	187	162	197	206		
	110	198	206	197	187	189				
	118	182		179	167	168				
	108				182					
Mean	104	159	174	174	178	187	204	196	183	178
±SEM	±7	±2	±9	±11	±10	±7	±7	±7	±18	±14

APPENDIX D-III

TABLE 7-Raw Data for Total RNA Content, Concentration and RNA to DNA Ratio of the Different Groups of Animals

	BC	C14	AN14	A14	C25	AN25	A25	C33	AN33	A33
TOTAL RNA CONTENT (ugm)	826	1109	1276	1325	1535	----	1330	1356	1464	1239
	633	1519	1637	----	1709	1708	1389	1395	1620	1596
	648	1324	1187	1408	----	1423	1262	1303	1123	1371
	610	----	0945	1355	1175	1102	0850	1139	1155	----
	---	0954	1092	----	1300	1080	0998	1059	1642	0830
	360	1095	1361	0721	1098	1508	1422	1013	1383	1316
	359	0940	1823	0950	1401	1657	1748	1334		
	552	1325	1352	1597	----	----				
	637	1657		1384	1731	----				
	---				----					
Mean	578	1240	1334	1249	1421	1413	1286	1228	1398	1270
±SEM	±55	±92	±101	±114	±94	±110	±111	±59	±91	±125
RNA CONC. (mg/gm)	3.52	1.64	1.98	2.39	2.15	----	1.75	2.02	2.72	2.22
	2.44	2.26	2.75	----	1.93	2.64	2.07	2.59	2.73	2.38
	2.62	2.03	2.44	2.38	----	1.91	1.93	2.00	1.72	2.08
	2.72	----	2.03	2.34	1.78	1.55	1.34	2.08	1.75	----
	----	1.82	1.65	----	1.67	1.77	1.52	1.62	2.73	1.88
	1.75	1.88	2.39	2.33	2.28	2.36	2.20	1.69	2.48	2.53
	1.96	1.61	2.88	2.51	2.90	2.66	2.40	2.41		
	2.50	1.77	2.91	1.51	----	----				
	2.44	2.65		2.78	1.93	----				
	----				----					
Mean	2.51	1.95	2.38	2.32	2.11	2.21	1.93	2.16	2.35	2.24
±SEM	±.20	±.13	±.16	±.15	±.12	±.19	±.15	±.15	±.20	±.12
RNA to DNA	1.39	1.01	----	2.15	1.56	----	2.12	1.65	1.50	1.43
	----	1.65	1.52	----	1.20	1.66	1.50	2.21	1.48	1.87
	1.21	1.08	1.46	1.54	----	1.45	1.74	1.53	1.20	1.53
	1.36	----	1.37	1.30	1.24	1.28	1.14	1.37	1.56	----
	----	0.81	1.33	----	1.47	1.58	1.48	1.26	2.35	1.07
	0.96	1.34	2.06	1.26	1.50	2.15	1.98	1.46	2.04	1.70
	1.18	1.34	2.57	1.29	1.82	2.33		1.83		
	1.20	1.51	2.31	2.23	----	----				
	1.34	2.14		2.17	2.16	----				
	----				----					
Mean	1.23	1.36	1.80	1.71	1.56	1.75	1.73	1.62	1.69	1.52
±SEM	±.08	±.15	±.19	±.17	±.13	±.17	±.14	±.12	±.17	±.13

TABLE 8-Raw Data for DNA Content and Concentration and Total Number of Nuclei of the Different Groups of Animals

APPENDIX D-IV

	BC	C14	AN14	A14	C25	AN25	A25	C33	AN33	A33
DNA CONTENT (ugm)	---	1101	----	----	0987	0940	0626	0824	0974	0865
	535	0923	1080	0915	1424	1027	0925	0630	1096	0852
	585	1230	0815	1042	1189	0984	0728	0853	0932	0897
	---	0884	0692	----	0949	0854	0745	0832	0741	----
	375	1173	0819	0574	0884	0686	0672	0841	0698	0775
	303	0819	0659	0738	0731	0694	0719	0686	0679	0781
	459	0702	0707	0715	0770	0711	0816	0732		
	391	0879	0584	0638	0782	0700				
	470	0775		0616	0803	0754				
					0864					
Mean ±SEM	464 ±37	943 ±61	765 ±61	748 ±65	938 ±69	817 ±46	747 ±37	771 ±33	853 ±70	834 ±24
DNA CONC. (mg/gm)	2.55	1.56	----	----	1.45	1.15	0.85	1.23	1.68	1.55
	----	1.37	1.81	1.60	1.67	1.59	1.38	1.17	1.74	1.33
	2.16	1.89	1.67	1.80	1.68	1.32	1.11	1.31	1.37	1.36
	2.60	1.36	1.48	----	1.46	1.21	1.12	1.52	1.12	----
	----	2.23	1.24	1.86	1.32	1.12	1.03	1.29	1.16	1.75
	1.82	1.40	1.16	1.17	1.11	1.10	1.11	1.16	1.22	1.50
	1.66	1.20	1.12	1.25	1.26	1.14	1.12	1.32		
	2.08	1.18	1.26	1.16	1.23	1.08				
	1.82	1.24		1.12	1.22	1.26				
	1.91				1.27					
Mean ±SEM	2.04 ±.13	1.49 ±.12	1.39 ±.10	1.41 ±.12	1.38 ±.06	1.26 ±.06	1.13 ±.07	1.34 ±.05	1.43 ±.13	1.50 ±.08
TOTAL NUMBER NUCLEI ($x10^6$)	96	176	---	099	159	152	101	133	157	140
	--	149	174	---	230	166	149	102	178	137
	86	198	132	148	192	159	117	138	150	145
	94	143	112	168	153	138	120	134	120	---
	--	189	132	---	143	111	108	136	113	125
	61	132	106	093	118	112	116	111	110	126
	49	113	114	119	124	115	132	118		
	74	142	094	115	126	113				
	63	125		103	130	122				
	76				139					
Mean ±SEM	75 ±6	152 ±10	123 ±10	121 ±10	151 ±11	132 ±22	121 ±6	124 ±5	138 ±11	135 ±4

TABLE 9-Raw Data for Weight per Nucleus, Hydroxyproline Content and Concentration of the Different Animal Groups

APPENDIX D-V

	BC	C14	AN14	A14	C25	AN25	A25	C33	AN33	A33
WEIGHT per NUCLEUS (mug)	2.43	4.01	----	5.58	4.27	4.88	7.33	4.73	3.42	3.98
	----	4.74	3.41	----	3.71	3.74	4.28	4.88	3.36	4.69
	2.86	3.29	3.70	4.01	3.68	4.32	5.20	4.44	4.34	4.55
	2.37	4.57	4.18	3.44	4.02	4.90	5.29	3.91	5.55	----
	----	2.78	5.02	----	4.47	5.53	6.04	4.47	5.35	3.54
	3.41	4.42	5.36	3.34	5.57	5.70	5.59	5.43	5.09	4.13
	3.74	5.17	5.55	5.31	4.94	5.44	5.55	4.69		
	2.99	5.28	4.94	4.98	5.07	5.72				
	3.41	5.00		5,34	5.10					
	3.24				4.90					
Mean ±SEM	3.12 ±.18	4.33 ±.28	4.59 ±.32	4.57 ±.36	4.57 ±.20	5.02 ±.22	5.61 ±.35	4.65 ±.18	4.52 ±.39	4.18 ±.21
HYDROXY-PROLINE CONTENT (ugm)	056	---	260	209	286	330	304	---	313	317
	---	365	276	---	374	312	281	270	353	290
	105	351	243	212	281	---	299	312	369	446
	059	364	214	302	---	398	279	374	384	---
	---	221	266	---	335	282	400	328	468	282
	066	269	274	161	331	317	306	384	347	302
	044	222	254	242	286	213	365	455		
	093	330	274	239	351	375				
	---	280		187	236	276				
	080				317					
Mean ±SEM	072 ±8	300 ±21	258 ±7	222 ±17	311 ±14	313 ±21	319 ±17	354 ±27	372 ±22	327 ±30
HYDROXY-PROLINE CONC. (mg/gm)	0.29	----	0.40	0.37	0.40	0.44	0.41	----	0.58	0.56
	----	0.54	0.46	----	0.43	0.50	0.44	----	0.59	0.45
	0.42	0.53	0.49	0.35	0.39	----	0.48	0.47	0.56	0.67
	0.26	0.56	0.46	0.52	----	0.59	0.44	0.71	0.58	----
	----	0.42	0.40	----	0.53	0.47	0.61	0.54	0.72	0.64
	0.32	0.46	0.48	0.52	0.50	0.48	0.44	0.64	0.57	0.58
	0.24	0.38	0.40	0.40	0.44	0.32	0.50	0.76		
	0.42	0.44	0.59	0.40	0.52	0.58				
	----	0.46		0.34	0.36	0.46				
	0.33				0.46					
Mean ±SEM	0.31 ±.03	0.47 ±.02	0.46 ±.02	0.42 ±.03	0.46 ±.02	0.49 ±.03	0.48 ±.03	0.63 ±.05	0.62 ±.03	0.58 ±.04

APPENDIX E

TWO-WAY ANOVA

AND

STUDENT NEWMAN-KEULS

FOR

EACH DEPENDENT VARIABLE

TABLE 10-Two-Way ANOVA Table and Student Newman-Keuls Post Hoc Test on Significant Effects for BODY WEIGHT.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
BODY WEIGHT	A	148229.938	2	74114.938	41.343	0.000
	B	35886.707	2	17943.352	10.009	0.000
	AB	4788.625	4	1197.156	0.668	0.617
	ERROR	95011.438	53	1792.668		
	TOTAL	284192.625	61	4658.895		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	416(gm)	369(gm)	339(gm)
25-week Group Mean	500(gm)	489(gm)	451(gm)
33-week Group Mean	447(gm)	480(gm)	509(gm)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	120(gm)	416(gm)	500(gm)	509(gm)
Anaerobic Group Mean	120(gm)	369(gm)	489(gm)	480(gm)
Aerobic Group Mean	120(gm)	339(gm)	451(gm)	447(gm)

[a]The Lines (———)show significant differences when the numbers are underlined with seperate lines.

[b]Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 11- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for HEART WEIGHT.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
HEART WEIGHT	A	646178,188	2	323089.063	7.302	0.000
	B	12444.824	2	6222.410	14.110	0.000
	AB.	29660.875	4	7415.219	0.272	0.763
	ERROR	1419656.00	62	22897.000		
	TOTAL	2118109.000	70	30258.699		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	1121(mg)	1048(mg)	1040(mg)
25-week Group Mean	1294(mg)	1297(mg)	1274(mg)
33-week Group Mean	1221(mg)	1214(mg)	1249(mg)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	473(mg)	1121(mg)	1294(mg)	1221(mg)
Anaerobic Group Mean	473(mg)	1048(mg)	1297(mg)	1214(mg)
Aerobic Group Mean	473(mg)	1040(mg)	1274(mg)	1249(mg)

[a]The Lines (——) show significant differences when the numbers are underlined with separate lines.
[b]Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 12- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for L.VENTRICULAR WEIGHT.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
LEFT VENTRICULAR WEIGHT	A	74508.563	2	37254.281	5.545	0.001
	B	5355.051	2	2677.525	10.443	0.000
	AB	13016.500	4	3254.125	0.912	0.000
	ERROR	189076.438	53	3567.480		
	TOTAL	281229.813	61	4610.324		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	674(mg)	632(mg)	596(mg)
25-week Group Mean	710(mg)	684(mg)	700(mg)
33-week Group Mean	617(mg)	640(mg)	632(mg)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	251(mg)	674(mg)	710(mg)	617(mg)
Anaerobic Group Mean	251(mg)	632(mg)	684(mg)	640(mg)
Aerobic Group Mean	251(mg)	596(mg)	700(mg)	632(mg)

[a]The lines (———) show significant differences when the numbers are underlined with separate lines.

[b]Source B also represent the One-Way ANOVA for growth (i.e.) between 5 weeks (BC) to 33 weeks).

TABLE 13- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for TOTAL PROTEIN.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
TOTAL PROTEIN	B	4188.852	2	2094.420	6.625	0.003
	A	860.276	2	430.138	1.361	0.265
	BA	389.266	4	97.316	0.308	0.871
	ERROR	16755.316	53	316.138		
	TOTAL	22145.789	61	363.046		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	143(mg)	133(mg)	132(mg)
25-week Group Mean	160(mg)	151(mg)	152(mg)
33-week Group Mean	149(mg)	151(mg)	149(mg)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	51(mg)	143(mg)	160(mg)	149(mg)
Anaerobic Group Mean	51(mg)	133(mg)	151(mg)	151(mg)
Aerobic Group Mean	51(mg)	132(mg)	152(mg)	149(mg)

[a] The Lines (——) show significant differences when the numbers are underlined with separate lines.
[b] Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 14 - Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for PROTEIN CONC..

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
PROTEIN CONCENTRATION	B	7424.836	2	3712.415	7.488	0.001
	A	181.886	2	90.943	0.183	0.833
	BA	1154.363	4	288.591	0.582	0.677
	ERROR	24293.773	49	495.701		
	TOTAL	33199.402	57	582.446		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	225(mg/gm)	236(mg/gm)	227(mg/gm)
25-week Group Mean	239(mg/gm)	233(mg/gm)	230(mg/gm)
33-week Group Mean	264(mg/gm)	251(mg/gm)	263(mg/gm)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	214(mg/gm)	225(mg/gm)	239(mg/gm)	264(mg/gm)
Anaerobic Group Mean	214(mg/gm)	236(mg/gm)	233(mg/gm)	251(mg/gm)
Group Mean	214(mg/gm)	227(mg/gm)	230(mg/gm)	263(mg/gm)

[a] The lines (———) show significant differences when the numbers are underlined with separate lines.
[b] Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 15- Two-Way ANOVA and Student Newman-Keuls Post Hos Test on Significant Effects for TOTAL RNA.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
TOTAL RNA	B	130623.688	2	65311.844	0.877	0.423
	A	150935.250	2	75467.625	1.014	0.371
	BA	112846.375	4	28211.594	0.379	0.823
	ERROR	3424894.000	46	74454.188		
	TOTAL	3795595.000	54	70288.750		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	1240(ugm)	1334(umg)	1249(ugm)
25-week Group Mean	1421(ugm)	1413(ugm)	1286(ugm)
33-week Group Mean	1228(ugm)	1398(ugm)	1270(ugm)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	578(ugm)	1240(ugm)	1421(ugm)	1228(ugm)
Anaerobic Group Mean	578(ugm)	1334(ugm)	1413(ugm)	1398(ugm)
Aerobic Group Mean	578(ugm)	1249(ugm)	1286(ugm)	1270(ugm)

[a] The lines (————) show significant differences when the numbers are underlined with separate lines.
[b] Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 16- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for RNA CONCENTRATION.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
RNA CONCENTRATION	B	306464.188	2	153232.063	0.903	0.412
	A	600242.061	2	300121.000	1.769	0.182
	BA	533745.938	4	133436.438	0.786	0.540
	ERROR	7804509.000	46	169663.188		
	TOTAL	9295180.000	54	172132.938		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	1.95(mg/gm)	2.38(mg/gm)	2.32(mg/gm)
25-week Group Mean	2.11(mg/gm)	2.21(mg/gm)	1.93(mg/gm)
33-week Group Mean	2.16(mg/gm)	2.35(mg/gm)	2.24(mg/gm)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	2.51(mg/gm)	1.95(mg/gm)	2.11(mg/gm)	2.16(mg/gm)
Anaerobic Group Mean	2.51(mg/gm)	2.38(mg/gm)	2.21(mg/gm)	2.35(mg/gm)
Aerobic Group Mean	2.51(mg/gm)	2.32(mg/gm)	1.93(mg/gm)	2.24(mg/gm)

[a] The lines(———)show significant differences when the numbers are underlined with separate lines.
[b] Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 17- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for RNA to DNA RATIO.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
RNA to DNA RATIO	B	0.042	2	0 021	0.126	0.881
	A	0.522	2	0.261	1.556	0.222
	BA	0.357	4	0.089	0.533	0.712
	ERROR	7.885	47	0.168		
	TOTAL	8.811	55	0.160		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	1.36	1.80	1.71
25-week Group Mean	1.56	1.75	1.73
33-week Group Mean	1.62	1.69	1.52

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	1.23	1.36	1.56	1.62
Anaerobic Group Mean	1.23	1.80	1.75	1.69
Aerobic Group Mean	1.23	1.71	1.73	1.52

[a]The lines (———)show significant differences when the numbers are underlined with separate lines.
[b]Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 18- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for PROTEIN/DNA RATIO.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
PROTEIN TO DNA RATIO	B	0.005	2	0.002	2.887	0.066
	A	0.001	2	0.000	0.480	0.622
	BA	0.004	4	0.001	1.096	0.369
	ERROR	0.040	47	0.001		
	TOTAL	0.050	55	0.001		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	159	174	174
25-week Group Mean	178	187	204
33-week Group Mean	196	183	178

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	104	159	178	196
Anaerobic Group Mean	104	174	187	183
Group Mean	104	174	204	178

[a]The lines (————)show significant differences when the numbers are underlined with separate lines.
[b]Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 19 - Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for TOTAL DNA.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
TOTAL DNA	B	82413.563	2	41206.781	1.607	0.210
	A	265107.750	2	132553.875	2.170	0.030
	BA	151229.000	4	37807.250	1.475	0.223
	ERROR	1358755.000	53	25636.887		
	TOTAL	1850883.000	61	30342.344		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	943(ugm)	765(ugm)	748(ugm)
25-week Group Mean	938(ugm)	817(ugm)	747(ugm)
33-week Group Mean	771(ugm)	853(ugm)	834(ugm)

Source B

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	464(ugm)	943(ugm)	938(ugm)	771(ugm)
Anaerobic Group Mean	464(ugm)	765(ugm)	817(ugm)	853(ugm)
Aerobic Group Mean	464(ugm)	748(ugm)	747(ugm)	834(ugm)

[a]The lines (——) show significant differences when the numbers are underlined with separate lines.

TABLE 20 - Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for DNA CONCENTRATION.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
DNA CONCENTRATION	B	8920.344	2	4460.172	0.180	0.836
	A	185062.813	2	92531.375	3.730	0.030
	BA	190990.750	4	47747.703	1.925	0.118
	ERROR	1438895.000	58	24808.531		
	TOTAL	1823931.000	66	27635.316		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	1.49(mg/gm)	1.39(mg/gm)	1.41(mg/gm)
25-week Group Mean	1.38(mg/gm)	1.26(mg/gm)	1.13(mg/gm)
33-week Group Mean	1.34(mg/gm)	1.43(mg/gm)	1.50(mg/gm)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	2.04(mg/gm)	1.49(mg/gm)	1.38(mg/gm)	1.34(mg/gm)
Anaerobic Group Mean	2.04(mg/gm)	1.39(mg/gm)	1.26(mg/gm)	1.43(mg/gm)
Aerobic Group Mean	2.04(mg/gm)	1.41(mg/gm)	1.13(mg/gm)	1.50(mg/gm)

[a]The lines(———)show significant differences when the numbers are underlined with separate lines.

[b]Source B represent also the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 21- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for NUMBER OF NUCLEI.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
TOTAL NUMBER OF NUCLEI	B	232.052	2	116.026	0.180	0.836
	A	4814.465	2	2407.232	3.730	0.030
	BA	4968.668	4	1242.167	1.925	0.118
	ERROR	37432.219	58	645.383		
	TOTAL	47448.992	66	718.924		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	152($\times 10^6$)	123($\times 10^6$)	121($\times 10^6$)
25-week Group Mean	151($\times 10^6$)	132($\times 10^6$)	121($\times 10^6$)
33-week Group Mean	124($\times 10^6$)	138($\times 10^6$)	135($\times 10^6$)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	75($\times 10^6$)	152($\times 10^6$)	151($\times 10^6$)	124($\times 10^6$)
Anaerobic Group Mean	75($\times 10^6$)	123($\times 10^6$)	132($\times 10^6$)	138($\times 10^6$)
Aerobic Group Mean	75($\times 10^6$)	121($\times 10^6$)	121($\times 10^6$)	135($\times 10^6$)

[a]The lines (———)show significant differences when the numbers are underlined with separate lines.

[b]Source B represent also the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 22- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for WEIGHT PER NUCLEUS.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
WEIGHT PER NUCLEUS	B	4.451	2	2.226	3.728	0.030
	A	1.420	2	0.710	1.189	0.312
	BA	4.020	4	1.005	1.684	0.166
	ERROR	34.624	58	0.597		
	TOTAL	44.492	66	0.674		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	4.34(mug)	4.59(mug)	4.57(mug)
25-week Group Mean	4.57(mug)	5.02(mug)	5.61(mug)
33-week Group Mean	4.65(mug)	4.52(mug)	4.18(mug)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	3.12(mug)	4.33(mug)	4.57(mug)	4.65(mug)
Anaerobic Group Mean	3.12(mug)	4.59(mug)	5.02(mug)	4.52(mug)
Aerobic Group Mean	3.12(mug)	4.57(mug)	5.61(mug)	4.18(mug)

[a]The lines (———)show significant differences when the numbers are underlined with separate lines.

[b]Source B represent also the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 23- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for HYDROXYPROLINE.

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
TOTAL HYDROXYPROLINE	B	73149.813	2	36574.906	14.738	0.000
	A	12896.941	2	6448.469	2.598	0.085
	BA	18473.813	4	4618.453	1.86	0.133
	ERROR	114157.063	46	2481.675		
	TOTAL	222033.750	54	4111.734		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	300(mg)	258(mg)	222(mg)
25-week Group Mean	311(mg)	313(mg)	319(mg)
33-week Group Mean	354(mg)	372(mg)	327(mg)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	72(mg)	300(mg)	311(mg)	354(mg)
Anaerobic Group Mean	72(mg)	258(mg)	313(mg)	372(mg)
Aerobic Group Mean	72(mg)	222(mg)	319(mg)	327(mg)

[a]The Lines (——)show significant differences when the numbers are underlined with separate lines.
[b]Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).

TABLE 24- Two-Way ANOVA and Student Newman-Keuls Post Hoc Test on Significant Effects for HYDROXYPROLINE CONC..

Variable	Source	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio	Probability
HYDROXYPROLINE CONCENTRATION	B	242281.875	2	121140.938	19.987	0.000
	A	11395.844	2	5697.922	0.940	0.398
	BA	15990.188	4	3997.547	0.660	0.623
	ERROR	278810.188	46	6061.090		
	TOTAL	561297.125	54	10394.391		

Student Newman-Keuls Post Hoc Test:[a]

Source A

	Control	Anaerobic	Aerobic
14-week Group Mean	474(mg/gm)	463(mg/gm)	417(mg/gm)
25-week Group Mean	458(mg/gm)	485(mg/gm)	480(mg/gm)
33-week Group Mean	628(mg/gm)	620(mg/gm)	583(mg/gm)

Source B[b]

	5 weeks	14 weeks	25 weeks	33 weeks
Control Group Mean	314(mg/gm)	474(mg/gm)	458(mg/gm)	628(mg/gm)
Anaerobic Group Mean	314(mg/gm)	463(mg/gm)	485(mg/gm)	620(mg/gm)
Aerobic Group Mean	314(mg/gm)	417(mg/gm)	480(mg/gm)	583(mg/gm)

[a] The lines (——) show significant differences when the numbers are underlined with separate lines.
[b] Source B also represent the One-Way ANOVA for growth (i.e. between 5 weeks (BC) to 33 weeks).